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ANTAGONISTIC ACTIVITY OF MICRO-ORGANISMS IN THE CONTROL OF BARLEY SMUTS[1]

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ABSTRACT

Covered smut of barley (*Ustilago hordei*) was controlled by soaking the seed in broth cultures of *Pseudomonas viscosa* or of a filamentous yeast at room temperature for 50 hours, or at 86° F. for 40 hours. Soaking the seed under similar conditions in a culture of *Bacillus subtilis* or in water did not give satisfactory control.

Control of loose smut of barley (*U. nuda*) was secured by soaking infected seed in a broth culture of *P. viscosa*, or in water, at room temperature for 60 hours. Control was only slightly less effective when seeds were soaked at 76° F. for 40 hours or 86° F. for 22 hours. Soaking at 66° F. for 50 hours resulted in unsatisfactory control, but the *P. viscosa* treatment was somewhat more effective than the water treatment.

INTRODUCTION

During the past nine years a number of reports have been published dealing with the control of seed-borne diseases by treatment of infected seed directly or indirectly with antifungal micro-organisms. In most of these studies antibiotic substances were used. Only a limited amount of work has been done with cultures of organisms that produced these antibiotics. An *in vitro* study by Timonin (10) showed that patulin inhibited the germination of the spores and the growth of the mycelium of *Ustilago tritici*. However, seed treatment with this antibiotic failed to control loose smut of wheat. Paulus and Starr (7) attempted to control loose smut of barley by soaking the seed in solutions of eight different antibiotic substances. None of the treatments gave satisfactory results, but streptomycin reduced the incidence of diseased plants by one-half. In 1951 Henry *et al.* (4) published the results of an experiment in which satisfactory control of covered smut of oats was secured by soaking the seed in a weak solution of actidione in water. The same treatment gave partial control of covered smut of barley. Bunt of wheat was controlled also, but injury to the wheat seed was severe. In a later experiment (5) good control, with very little seed injury was achieved with actidione carried either in water or in powdered clay. Leben *et al.* (6) tested the effectiveness of several forms

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of helixin and of crystalline antimycin, as seed treatments for the control of oat smut and bunt of wheat. Certain variations of these treatments proved effective against both smut diseases.

Published reports of studies on the treatment of infected seed with cultures of micro-organisms are not numerous. Darpoux and Faivre-Amiot in 1950 (2) reported the control of a seedling disease of tobacco caused by *Pseudomonas tabaci*, by soaking the infected seed in the broth culture of an actinomycete. A year later (3) the above authors and Leblanc demonstrated that bunt of wheat can be controlled by soaking the seed in broth cultures of several different fungi. Some of these treatments interfered with the germination of the wheat but others did not. Recently Schroeder (9) showed that *Helminthosporium sativum* and *Gibberella zeae* were partially effective in controlling bunt of wheat.

In the course of experimentation at this laboratory certain micro-organisms antagonistic to some of the fungi that cause root rot of cereals were found to be also antagonistic to numerous other fungi. The broad range of the micro-organisms and the intense antagonism exhibited by them suggested the desirability of testing the capacity of these cultures to control certain plant diseases. The present study represents an attempt to assess their effectiveness in the control of covered and loose smuts of barley.

MATERIALS AND METHODS

Samples of seed of different varieties of barley commonly grown in Saskatchewan were used. These samples were naturally infected with either *Ustilago hordei*, the causal organism of covered smut, or with *U. nuda*, the causal organism of loose smut.

The micro-organisms used included a filamentous yeast designated as A16, and two species of bacteria, *Bacillus subtilis* and *Pesudomonas viscosa*. These three organisms were isolated locally; *P. viscosa* was described recently by Chinn (1). In the present study their antibiotic activities were measured against *U. hordei* and *U. nuda* by a modification of the spot-inoculation technique (1). A yeast-extract mineral agar of the following composition was used: glucose, 20 gm.; yeast extracts, 5 gm.; ammonium sulphate, 2 gm.; dipotassium phosphate, 1 gm.; magnesium sulphate, 0.01 gm., tap-water, 800 ml.; soil extract, 200 ml.; and agar, 15 gm. A16 and *P. viscosa* were spot-inoculated on the same plates but *B. subtilis*, because of its spreading nature, was spot-inoculated by itself on separate plates. After incubation at 76° F. for two days each plate was seeded with spores from barley heads infected with *U. hordei* or *U. nuda*. After a further incubation at 76° F. for 24 hours, examination for antagonism was made by measuring the width of the zones of inhibition.

Cultures were prepared by growing the test organisms in a broth of the same composition as the medium just described, with the agar omitted. Incubation was at approximately 72° F. for four days. The infected seed was soaked for definite periods of time in the broth culture, then air-dried before being sown.

The investigation included experiments in the greenhouse and in field plots. In the recording of yield, counts of plants were taken of greenhouse crops whereas counts of heads were taken in the field plots.

EXPERIMENTAL RESULTS

Antibiotic activities of the three micro-organisms

In the study of the antibiotic activities of *P. viscosa*, *B. subtilis*, and A16 on *U. hordei* and *U. nuda* it was found that the growth of *P. viscosa* inhibited to a relatively high degree the germination and development of the chlamydospores of *U. hordei* or *U. nuda*. *B. subtilis* on the other hand did not inhibit the germination of the two fungi although it did retard their further development somewhat. A16 had no effect against either of the fungi.

The Control of Covered Smut

In a preliminary trial performed in the greenhouse, barley seed infected with *U. hordei* was soaked in cultures of *P. viscosa*, *B. subtilis*, or A16, or in water at room temperature for 40 hours. Subsequently 50 seeds from each treatment were sown in crocks. The results showed that treatment with *P. viscosa* eliminated most of the disease and that treatment with A16 probably had some therapeutic value. Treatment with *B. subtilis* and the water soak treatment failed to control the smut.

Three trials for the control of covered smut were conducted in the field. The same seed sample was used in all these trials. In the first trial the seed was soaked in broth cultures of *P. viscosa*, *B. subtilis*, or A16 at room temperature for 50 hours. The seed from each treatment was sown in ten 8-foot rows, at the rate of 120 seeds per row. The results of this trial are shown in Table 1. Some comparable data on the water-soak treatment and a dry check from Table 2 are included.

TABLE 1.—EFFECT OF SOAKING BARLEY SEED IN BROTH CULTURES OF THREE MICRO-ORGANISMS AT ROOM TEMPERATURE FOR 50 HOURS ON THE CONTROL OF COVERED SMUT

Treatment	Total number of heads	Percentage smutted heads
P. viscosa	1263	0
B. subtilis	1272	15.6
A16 (yeast)	966	0
Water soak		8.8
Dry check		10.6

The data show that soaking the seed in a culture of *P. viscosa* eliminated the smut entirely; the yield of healthy heads following this treatment was superior to the yields following the other two treatments. The treatment with *B. subtilis* did not reduce the amount of smut; if anything, it caused an increase in the percentage of smutted heads. Treatment with the yeast gave complete control of the smut but depressed the yield through reduced emergence.

In the second field trial, treatment with *P. viscosa* was compared with the Panogen*, Mema†, and water-soak treatments. A dry check was

*Panogen—Panogen Incorporated.
†Mema—Chipman Limited.

TABLE 2.—A COMPARISON OF THE EFFECT OF SOAKING BARLEY SEED IN BROTH CULTURE OF *Pesudomonas viscosa* AND WATER AT ROOM TEMPERATURE FOR 50 HOURS WITH TWO CHEMICAL TREATMENTS ON THE CONTROL OF COVERED SMUT

Treatment	Total number of heads	Percentage of heads smutted[1]	Yields[2]
P. viscosa	3246	0.4	93
Water soak	3873	8.8	102
Mema	4177	0.4	120
Panogen	4286	0.09	123
Dry check	4078	10.6	100

[1] Some loose smut occurred in this experiment but it was not included in the calculations.
[2] The number of healthy heads expressed as a percentage of the number of healthy heads in the check.

included. The water-soak treatment and the treatment with *P. viscosa* were applied at room temperature for 50 hours. The two organic mercurials were applied as liquids at the recommended rates. Five plots, each consisting of five 8-foot rows sown at the rate of 120 seeds per row, were devoted to each treatment. The plots were arranged in the form of a Latin square. The data presented in Table 2 show that the treatment with *P. viscosa* gave good control of covered smut, reducing it to about the same level as the two mercuric fungicides, but apparently it caused enough injury to the seed to lower the yield somewhat. The water-soak treatment caused only a slight reduction in the amount of smut.

A third field trial was conducted the following year employing broth cultures of the same three micro-organisms; incubation temperature was increased to 86° F. and the seed was treated for 20 and 40 hours. These treatments were compared with the water-soak and Panogen treatments. Because of shortage of seed only five 8-foot rows were devoted to each treatment.

TABLE 3.—A COMPARISON OF THE EFFECT OF SOAKING BARLEY SEED IN BROTH CULTURES OF THREE MICRO-ORGANISMS AND IN WATER AT 86° F. FOR 20 AND 40 HOURS WITH THE PANOGEN TREATMENT ON THE CONTROL OF COVERED SMUT

Treatment	Length of treatment in hours	Total number of heads	Percentage of heads smutted	Yields[1]
P. viscosa	20	589	2	135
B. subtilis	20	637	26	110
A16 (a yeast)	20	604	22	109
Water soak	20	608	10	127
P. viscosa	40	546	0	127
B. subtilis	40	570	13	115
A16 (a yeast)	40	353	0	82
Water soak	40	453	2	103
Panogen	—	649	0	150
Dry check	—	550	22	100

[1] The number of healthy heads expressed in percentage of the number of healthy heads in the check.

The data presented in Table 3 show that none of the treatments when applied for 20 hours eliminated the smut completely. However, treatment with the culture of *P. viscosa* gave almost complete control. When the treatments were applied for 40 hours, control was complete after treatment with the cultures of *P. viscosa* and the yeast, and almost complete after the water-soak treatment. Treatment with *B. subtilis* gave partial control. Panogen controlled the disease completely. Treatment with the *P. viscosa* culture resulted in a high yield, but treatment with the yeast culture depressed the yield.

The Control of Loose Smut

In the experiments on the control of loose smut with cultures of microorganisms only *P. viscosa* was employed. Three trials, two in the greenhouse and one in the field, were conducted.

In the first greenhouse trial the seed was soaked in a culture of *P. viscosa* or water at room temperature for periods of 24, 48, and 60 hours. The data presented in Table 4 show that at room temperature the degree of control of loose smut of barley depended on the length of the soak periods. Control was absolute when the period of soaking was 60 hours but the effectiveness decreased when shorter periods were employed. However, with these shorter periods, somewhat better control was obtained when the seed was treateed with *P. viscosa* than with water.

TABLE 4.—THE EFFECT OF SOAKING BARLEY SEED, FOR VARIOUS PERIODS AT ROOM TEMPERATURE IN A CULTURE OF *Pseudomonas viscosa* OR IN WATER, ON THE CONTROL OF LOOSE SMUT

Treatment	Length of soak in hours	Total number of plants	Percentage of plants smutted
P. viscosa	24	45	13.3
P. viscosa	48	45	4.4
P. viscosa	60	38	0.0
Water	24	48	33.3
Water	48	45	6.7
Water	60	42	0
Dry check	—	47	21.3

In the field trial, soaking the seed in a broth culture of *P. viscosa* at 86° F. for 24 hours was compared with the hot water (8) and the Spergon (11) treatments. Seed from these treatments and a dry check were sown in plots, each consisting of five 8-foot rows, replicated four times. The results presented in Table 5 show that all the treatments gave satisfactory control of the disease but varied considerably in their effect on the yield of the barley. The best yield was obtained with the culture treatment; the Spergon treatment resulted in a poor yield.

TABLE 5.—THE COMPARATIVE EFFICIENCY OF THREE DIFFERENT TREATMENTS FOR THE CONTROL OF LOOSE SMUT OF BARLEY

Treatment	Total number of heads	Percentage of heads smutted	Yields[1]
P. viscosa	3963	0.08	118
Hot water	3604	0.03	107
Spergon	1696	0.0	51
Dry check	4058	17.3	100

[1] The number of healthy heads expressed as a percentage of the number of healthy heads in the check.

TABLE 6.—COMPARATIVE EFFICIENCY OF SOAKING INFECTED SEED IN A CULTURE OF *P. viscosa* AT DIFFERENT TEMPERATURES FOR VARIOUS PERIODS ON THE CONTROL OF LOOSE SMUT OF BARLEY

Soak	Treatment		Total heads	Per cent smutted	Yields[1]
	Time	Temperature			
P. viscosa	50 hr.	66° F.	297	7.4	108
Water	50 hr.	66° F.	306	11.1	107
P. viscosa	40 hr.	76° F.	301	0.7	117
Water	40 hr.	76° F.	282	0.7	110
P. viscosa	22 hr.	86° F.	292	1.0	113
Water	22 hr.	86° F.	285	1.4	110
Check: untreated			299	14.7	100

[1] The number of healthy heads expressed as a percentage of the number of healthy heads in the check.

In the second greenhouse trial the efficiency of soaking the seed in a broth culture of *P. viscosa* at different temperatures for various periods was compared with that of soaking in water under similar conditions. Treatment temperatures and periods of 66° F. for 50 hours, 76° F. for 40 hours, and 86° F. for 22 hours were used. The results presented in Table 6 show that at the two higher temperatures there was very little difference in the efficiency of the two treatments. However, at 66° F. treatment with the broth culture of *P. viscosa* gave slightly better control than treatment with water.

DISCUSSION

The results of the experiments presented in this paper suggest that treatment of seed with cultures of antagonistic micro-organisms as distinct from antibiotic substances may offer possibilities in the control of certain cereal smuts. Henry *et al.* (4) demonstrated that the antibiotic, actidione, gave only partial control of covered smut of barley whereas, in the present study, treatment with cultures of either *P. viscosa* or A16 gave complete control of this disease. It should be stressed that only three species of micro-organisms were used and two of them gave favourable results. Since limitless numbers of species of micro-organisms are available for such experimentation, the possibilities of the cultural type of seed treatment should be further assessed not only for the control of covered smut of barley but also for the control of numerous other seed-borne plant diseases. In conjunction with this type of seed treatment there is also the possibility that the outcome may be influenced by the composition of the broth medium.

According to the antibiotic spectrum obtained in this study, *U. hordei* was found to be inhibited by *P. viscosa* and restricted to some extent by *B. subtilis*, but not influenced by A16; yet both *P. viscosa* and A16 were effective in the seed treatment experiment. Why A16 showed no antagonism towards *U. hordei* in the *in vitro* study, but was found to be effective in the seed treatment test, should be further investigated.

The authors are aware of a number of experiments being made on the effectiveness of antibiotics for the control of loose smut. However, no encouraging results have been reported. In the present study both the broth culture and the water-soak treatments, under certain temperature and time conditions, were found to be effective in the control of the disease. Since the culture treatment gave good control it was thought that the antibiotic substance(s) produced by *P. viscosa* was responsible for the result. Similar deductions can be made also on the favourable effect of the metabolic substance(s) produced by the natural flora of the seed during the water-soak treatment. However, more recent experiments by the authors and those reported by Leben *et al.* (6) suggest that micro-organisms played very little or no part in the control. Experiments are being conducted to elucidate the mechanism involved.

From a practical standpoint the relative cost and convenience of different treatments are matters of primary importance. Although the culture treatments gave good control of covered smut, technical difficulties and perhaps high cost of these treatments will prevent their being used on the farm, especially when low cost mercurial fungicides are available. In the case of loose smut, the water-soak treatment was just as effective as the culture treatment, so that there is no need to advocate the use of the latter. However, there may be a place for the culture treatment; seed infected with both *U. hordei* and *U. nuda* needs only this treatment, whereas currently used fungicides are not effective against *U. nuda* and the water-soak treatment is only partially effective against *U. hordei*.

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WOOL GROWTH IN MATURE RANGE EWES AS AFFECTED BY STAGE AND TYPE OF PREGNANCY AND TYPE OF REARING[1]

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ABSTRACT

Bi-monthly wool production from measured areas (4 sq. cm.) tattooed on the shoulders of mature range ewes was analysed to determine the effects of stage and type of pregnancy and type of rearing on weight of clean wool, fibre length, fibre thickness, and density of fibres per unit of skin area. Data were obtained from ewes on a relatively high plane of nutrition (10-13 per cent protein) and from those on a fairly low plane (7 per cent protein until 6 weeks before lambing and 10 per cent thereafter).

Weight of clean wool, average fibre length, and density of fibre per unit of skin area were significantly higher from ewes with singles than from ewes with twins during pregnancy and lactation. The level of nutrition determined the stage of pregnancy at which significant differences began to occur.

Bi-monthly weights of clean wool within ewe groups indicated that both early and advanced pregnancy, and lactation significantly reduced growth. For density of fibre a reduction was induced earlier on the lower level of nutrition than on the higher level.

Average fibre thickness was significantly different (greater) between ewes with singles and ewes with twins during advanced pregnancy on the lower plane of nutrition. Within ewe groups early and late pregnancy, and lactation, significantly decreased average fibre thickness on both levels of feeding.

Total production of clean wool (8 months' growth) from the measured areas of the ewes with singles was 19 per cent greater ($p < \cdot 01$) than that from the ewes with twins on the lower plane of nutrition. Although this difference amounted to 11 per cent on the higher plane of nutrition it was not significant.

Irrespective of the level of feeding, the average production values within types of pregnancy were reduced to similar levels by the end of lactation.

INTRODUCTION

Growth of the wool fibre is a continuous process which is regulated by the inherited characteristics and the general condition of the sheep. Consequently, the amount of wool grown may be influenced markedly by changes in breeding (11), feeding practices (13, 14), age (9), disease (12), exposure to storms (6), and changes in temperature (4).

Burns (2, 3) reported that monthly wool growth (length of fibre) of Rambouillet and Hampshire ewes was reasonably uniform throughout the year. In this study neither pregnancy nor lactation appeared to have any pronounced effect on longitudinal growth. Jones *et al.* (8) found that barren Rambouillet ewes produced approximately 4 per cent more clean wool during a 12-month period, than ewes bearing lambs. Bell *et al.* (1) concluded from linear measurements that the rate of growth was influenced by parturition and early lactation but not by pregnancy. Slen and Whiting (13) in a study of bi-monthly wool production from measured shoulder areas over a 12-month period found that pregnancy and lactation markedly influenced wool growth. Johansson and Berg (7) and Stevens and Wright (16) obtained differences of 3 to 4 per cent in grease fleece weights between

[1] Contribution from the Division of Animal Husbandry, Experimental Farms Service.

ewes bearing single lambs and those bearing twins. Mason and Dassat (10) obtained a significant difference of 8 per cent between the two types of pregnancy in the coarse-wooled Langhe sheep of Italy. Turner (17) observed that ewes which reared a lamb had approximately 100 more fibres per square cm. than ewes which failed to raise a lamb. The author suggested that this probably resulted from the fact that ewes which failed to rear a lamb were in better condition and consequently had a greater skin area with a smaller fibre population than those which reared a lamb.

In view of the variations in wool production due to pregnancy and lactation reported previously by Slen and Whiting (13) further analyses were made on the bi-monthly wool production of mature range ewes to determine the influence of stage and type of pregnancy and type of rearing.

MATERIALS AND METHODS

The wool data analysed in this study were obtained from grade Corriedale and Romnelet ewes, 3 to 5 years of age, which were being used to determine the relation of level and source of protein to wool and lamb production. The ewes were bred so that lambing occurred in mid-April of each year.

Bi-monthly wool samples were obtained from measured areas on the sheep's skin to determine periodic wool growth. The sampling area (2 × 2 cm.) was tattooed on the flat portion of the shoulder immediately below the point of the scapula. Weight of clean wool, fibre length, fibre thickness, and density of fibres per square inch of skin area were determined from these samples (15).

The number of ewes from which data were collected and the periods of collection are shown in Table 1. There were not sufficient dry ewes or those giving birth to triplets for inclusion in these analyses.

For analysis the data first were separated on the basis of the ration fed. The wool samples obtained during 1949-50 and 1950-51 were from ewes receiving rations containing 10 to 13 per cent protein. The samples collected during the following three years (1951-54) were from ewes fed rations containing approximately 7 per cent protein until 6 weeks before lambing

TABLE 1.—THE NUMBER OF EWES SAMPLED DURING THE VARIOUS PERIODS OF PRODUCTION

Period of sampling	1949-51		1951-54	
	Ewes with singles	Ewes with twins	Ewes with singles	Ewes with twins
Oct.-Dec. (Breeding)	35	52	236	81
Dec.-Feb. (Early pregnancy)	35	52	236	81
Feb.-Apr. (Advanced pregnancy)	35	52	236	81
Apr.-June (Lactation)	33	13	220	61

and 10 per cent thereafter. The final separation was made on the basis of type of pregnancy and rearing. Comparisons between groups were made by testing the significance of differences between means by using the method of Goulden (5).

RESULTS AND DISCUSSION

The average bi-monthly weights of clean wool and related characteristics as affected by stage and type of pregnancy and method of rearing are shown in Table 2. The initial data on the various fibre characteristics showed no differences between the two types of pregnancy.

Weight of Clean Wool

Significant differences ($p < \cdot 01$) in the average bi-monthly weights of clean wool between ewes carrying and raising twins (hereafter referred to

TABLE 2.—WOOL PRODUCTION OF MATURE EWES AS AFFECTED BY STAGE AND TYPE OF PREGNANCY AND TYPE OF REARING

Period of sampling	1949-51[1]		1951-54[1]	
	Ewes carrying and raising single lambs	Ewes carrying and raising twin lambs	Ewes carrying and raising single lambs	Ewes carrying and raising twin lambs
	Av. weight of clean wool (mg.)			
Oct.-Dec.[4]	187·0[2]	182·9[2]	123·2[2]	121·9[2]
Dec.-Feb.	175·6[2]	163·0[2]	113·5[2 3]	104·8[2]
Feb.-Apr.	125·8[2 3]	101·3[2]	90·6[2 3]	68·3[2]
Apr.-June	81·8[3]	58·0	81·6[3]	51·8
Av. total production	570·1	512·2	409·0[3]	344·5
	Av. fibre length (mm.)			
Oct.-Dec.	22·2	22·7[2]	21·5[2]	21·5[2]
Dec.-Feb.	21·8[2]	22·2[2]	21·0[2]	20·4[2]
Feb.-Apr.	20·2[3]	19·3	19·4[3]	17·8
Apr.-June	19·0	18·5	19·1[3]	17·4
	Av. fibre thickness (μ)			
Oct.-Dec.	25·7[2]	26·2[2]	24·7[2]	25·3[2]
Dec.-Feb.	25·2[2]	25·4[2]	23·9[2]	24·0[2]
Feb.-Apr.	23·6[2]	22·9[2]	23·0[2 3]	22·0[2]
Apr.-June	20·8	19·8	22·0[3]	19·8
	Av. density of fibres per unit of skin area ('000)			
Oct.-Dec.	19·7	18·4	15·0[2]	14·9[2]
Dec.-Feb.	19·7[3]	17·8[2]	13·7	13·0
Feb.-Apr.	17·7[2 3]	15·7[2]	13·8[3]	12·8[2]
Apr.-June	15·0[3]	12·0	13·4[3]	11·5

[1] The ewes used during 1949-51 were on a higher plane of nutrition than those used during 1951-54 (see text).

[2] Significantly greater ($p < \cdot 01$) than the value immediately below.

[3] Significantly greater ($p < \cdot 01$) than the value immediately to the right.

[4] Data were collected the middle of each sampling month.

as "ewes with twins") and those carrying and raising singles (hereafter referred to as "ewes with singles") were obtained. On the higher plane of nutrition (1949-51) the effect of twinning became apparent during the advanced stages of pregnancy and continued during lactation. However, on the lower plane of nutrition (1951-54) significant differences ($p < \cdot 01$) also occurred during early pregnancy.

Bi-monthly wool production within groups was found to decrease significantly during early and advanced pregnancy and during lactation. This decrease was more pronounced in the ewes with twins than in those with singles at each stage of production. In a previous paper (13) the decrease during early pregnancy was attributed primarily to cold weather that prevailed during that time but the possibility of early pregnancy was suggested. On the basis of these data it would appear that early pregnancy was the primary factor in influencing wool production.

Although total clean wool production differed markedly the minimum level of production which occurred during lactation was the same regardless of the level of nutrition.

On the lower plane of nutrition the ewes with singles produced approximately 19 per cent more clean wool than those with twins. This difference in production was significant ($p < \cdot 01$). On the higher plane of nutrition, although the ewes with singles produced approximately 11 per cent more wool than those with twins, this difference was not significant. These differences are in general agreement with those obtained by Johansson and Berg (7), Stevens and Wright (16), and Mason and Dassat (10).

Fibre Length

In the group on the high plane of nutrition significant differences in average fibre length occurred between the ewes with twins and those with singles only during advanced pregnancy. In the ewes on the lower plane of nutrition the same effect was noted and also during lactation.

Within groups of the 1949-51 data increases in fibre length occurred until the end of the October-December period. Significant decreases ($p < \cdot 01$) occurred during the periods of early and late pregnancy (December-February and February-April) in the ewes with twins but only during advanced pregnancy in those with singles. In the ewes on the lower plane of nutrition significant decreases occurred during both the early and late stages of pregnancy. In all groups there was a small non-significant decrease during lactation.

Fibre Thickness

Type of pregnancy had no significant effect on the average fibre thickness when the ewes were maintained on rations containing 10 to 13 per cent protein. However, on the lower plane of nutrition the average fibre thickness of ewes with twins was significantly finer ($p < \cdot 01$) than those with singles during advanced pregnancy and during lactation.

Fibre thickness also was influenced markedly by the physiological changes in the ewe. Significant decreases were found during early and late pregnancy and during lactation in all groups.

Density of Fibres per Unit of Skin Area

Significantly lower values ($p < \cdot 01$) for the average density of fibres on the skin between the ewes with twins and those with singles occurred at both levels of nutrition during advanced pregnancy and during lactation. In addition, those ewes on the higher level of nutrition showed significant differences during early pregnancy.

Within ewe groups, early pregnancy had no effect on the density of fibres when the ewes were on the higher level of nutrition. However, in these groups the fibre density of the ewes with twins decreased significantly during advanced pregnancy and during lactation while those with singles did not change significantly until lactation began. In the ewes on the lower plane of nutrition the fibre densities of those with singles decreased significantly during early lactation but no further changes occurred. In the twin group significant decreases occurred during early pregnancy and during lactation. Although these results do not agree with those of Turner (17) the reason was probably due to differences in methods of determining fibre densities.

Wool Production of Ewes Carrying Twins but Raising Singles

The wool production data of ewes carrying twin lambs but raising singles are shown in Table 3. As would be expected, the average values for all fleece characters were similar to those from ewes with twins until the last sampling period. During that period the average values for the characters studied were somewhat, though not significantly, greater than those from ewes with twins. It is evident that the loss of one lamb reduced the demands on the ewe for milk production which resulted in an early response in wool growth.

TABLE 3.—WOOL PRODUCTION OF MATURE EWES[1] CARRYING TWIN LAMBS BUT RAISING A SINGLE (1949-51)

Period of sampling	Weight of clean wool (mgm.)	Fibre length (mm.)	Fibre thickness (μ)	Density of fibres per sq. in. ('000)
June-Aug.	115·2	19·3	23·3	18·6
Aug.-Oct.	166·3	21·8	24·8	19·1
Oct.-Dec.	180·9	22·5	26·0	18·7
Dec.-Feb.	160·6	22·0	25·3	18·0
Feb.-Apr.	97·9	19·0	22·5	15·5
Apr.-June	66·9	19·0	20·2	12·2

[1] 27 head.

It may be concluded that the stage and type of pregnancy and the type of rearing in mature range ewes had a marked influence on the amount of clean wool produced and also on the various fibre characteristics studied.

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FIELD TESTS WITH FUNGICIDES TO CONTROL DAMPING-OFF OF SCOTS PINE[1]

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ABSTRACT

Soil treatments with (*a*) formaldehyde, (*b*) acetic acid, (*c*) sulphuric acid, and (*d*) combined soil and seed treatment with Kolodust (sulphur and dichlone) failed to give consistent control against damping-off of Scots pine in trials in 1951 to 1953. Pelleting the seed with methyl cellulose and Dithane Z-78 (zineb), Orthocide 75 (captan), or Tersan (thiram) was tried in 1954 and gave good control. A combined treatment of seed pelleting and repeated soil applications with Tersan gave effective control early in the season but considerable seedling mortality occurred later. Pelleting with Dithane 14 (nabam) and Vancide 51 (sodium dimethyl dithiocarbamate and sodium 2-mercaptobenzothiozate) was ineffective or phytotoxic.

INTRODUCTION

Phytotoxicity endangers germinating seeds and young seedlings of conifers when such important fungicides as mercury compounds are applied (2, 3, 11). However, in recent trials (1, 8, 9) new fungicides of low phytotoxicity (2, 11) have controlled damping-off without injury to the host. The most extensively used fungicides in forest nurseries are formaldehyde and acidifying chemicals (3).

Many seed treatments have failed because fungicidal effectiveness did not last long enough (3, 9). The good control obtained by Berbee *et al.* (1) through pelleting the seed with methyl cellulose sticker and thiram is probably a result of the extended duration of effectiveness. Pelleting allows an extra large amount of fungicide to be bound to the seed.

In the present study, field tests were conducted with acidifying chemicals, formaldehyde, and some new fungicides chosen after a laboratory screening (11).

METHODS

The experiments were performed with Scots pine (*Pinus sylvestris* L.) at the Forest Nursery Station, Indian Head, Sask. The fungicides were tested in an old nursery in soil with fair loam content and slightly alkaline reaction, an environment known to favour damping-off. Hundreds of isolations were made from diseased seedlings, mainly in 1953 and 1954. The highly virulent pathogens *Rhizoctonia solani* Kühn (= *Corticium praticola* Kotila and *Pellicularia filamentosa* (Pat.) Rogers), *Pythium debaryanum* Hesse, and *Phytophthora cactorum* (Leb. and Cohn) Schroet. were found to be present. Judging from the incidence of various organisms in isolations, damping-off in the nursery was caused mainly by *P. debaryanum* in 1953 (incidence 25 per cent) and by *P. cactorum* in 1954 (11 per cent). Various other organisms were also commonly associated with damping-off but their importance as pathogens has not yet been thoroughly

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investigated and may be only small. These were species of the fungal genera *Fusarium*, *Cylindrocarpon*, *Alternaria*, *Phoma* and the nematode *Panagrolaimus* sp.

The trials were conducted during the growing seasons of 1951-1954, inclusive. The prevalence of *Pythium* in 1953 and *Phytophthora* in 1954 was probably favoured by the frequent heavy rains in these years. Possibly in other years other pathogens were more important.

The seed was broadcast and raked into plots 3.5 × 2.5 ft. at the rate of 3 gm. (approx. 400 seeds) per sq. ft. Each fungicide treatment was replicated in 5 blocks at random. In soil treatments the rates of application of fungicides per sq. ft. were: 2 per cent formaldehyde, 1 pint; 1 per cent acetic acid, 1.5 pints; 1 per cent sulphuric acid, 1 pint; Kolodust (83 per cent sulphur and 0.5 per cent dichlone), 12 gm.; Tersan (75 per cent thiram), 1 gm. Soil was treated on the day of sowing except that formaldehyde treatments were applied 3, 5, and 7 days earlier. Kolodust was mixed in a layer 4 inches thick; others were applied on the surface only. Kolodust soil treatment was supplemented by seed treatment (2 per cent of seed weight). Tersan soil treatment was supplemented by seed pelleting, and the soil applications were repeated every 5 weeks following seeding.

Seeds were pelleted by mixing 100 parts (by weight) of seed with 10 parts of 2.5 per cent aqueous cellulose solution, adding 20 parts of fungicide, and shaking again. The fungicides thus used were: Tersan, Orthocide 75 (75 per cent Captan), Dithane Z-78 (65 per cent Zineb), Dithane D-14 (19 per cent Nabam), and Vancide 51 (30 per cent of the mixture of sodium dimethyl dithiocarbamate and sodium 2-mercaptobenzothiozate). The two last mentioned fungicides are liquids; the others are dusts with sticking and dispersing powdery ingredients.

The procedure in making pellets was adopted after a laboratory experiment that gave the following results: In Petri dishes methyl cellulose pelleting decreased the germination of pine seed, especially if large amounts of cellulose solution were used (40 parts to 100 parts of seed). With smaller amounts, the germination was decreased only if the fungicide was left out of the pellet coat. The fungicide tried was Orthocide 75. This powdery fungicide may have made the pellet coat around the seed less firm, and thus in some way counteracted the decrease in germination.

RESULTS

Data on mortality during the growing season and the relative survival at the end of the season are presented in Tables 1 and 2. The mortality was mainly affected by two lethal factors, phytotoxicity of the treatments and damping-off caused by fungi.

In the formaldehyde plots considerable phytotoxicity occurred resulting in high germination failure shown in Table 1. Evidently the time between the treatment and seed sowing (3, 5 and 7 days) was too short and the seed itself was affected. Acetic acid also appeared phytotoxic in 1952.

Seed pelleting with Dithane D-14 and Vancide 51 (Table 2) may have been phytotoxic, but the cellulose effect may also explain the high germina-

TABLE 1.—MORTALITY OF SCOTS PINE DURING THE FIRST SEASON AS AFFECTED BY DAMPING-OFF AND FUNGICIDAL TREATMENTS. INDIAN HEAD, SASK., 1951 TO 1953

Treatment	Percentage germination failure†			Percentage seedling loss†			Relative survival (check = 1.0)		
	1951	1952	1953	1951	1952	1953	1951	1952	1953
Check	63	44	71	5.8	8.6	7.0	1.0	1.0	1.0
Kolodust	64	44	74	4.2	10.2	3.9	1.0	1.0	1.0
Acetic acid	61	57	64	3.6	25.8	10.4	1.1	0.4**	1.2
Sulphuric acid	64	37	57	5.1	8.5	7.9	1.0	1.1	1.6**
Formaldehyde (days before seeding)									
3	99	94	97	0.1	0.7	1.1	0.03**	0.1**	0.1**
5	88	82	97	1.3	1.5	1.3	0.3**	0.3**	0.1**
7	65	82	92	4.1	1.9	3.0	1.0	0.3**	0.2**

† In relation to the approximate number of seeds sown (3700 = 100).
** Survival significantly different from check at P = 0.01.

TABLE 2.—MORTALITY OF SCOTS PINE DURING THE FIRST SEASON AS AFFECTED BY DAMPING-OFF AND FUNGICIDAL TREATMENTS. INDIAN HEAD, SASK., 1954

Treatment	Percentage germination failure†	Percentage seedling loss†	Relative survival (check = 1.0)
Check	76	5.0	1.0
Tersan seed pellet and 6 soil treatments	64	7.1	1.5*
Tersan seed pellet	64	3.5	1.7**
Orthocide seed pellet	62	3.0	1.8**
Dithane Z-78 seed pellet	62	1.7	1.9**
Dithane D-14 seed pellet	77	1.7	1.1
Vancide 51 seed pellet	88	1.6	0.5*

† In relation to the approximate number of seeds sown (3700 = 100).
*, ** Survival significantly different from check at P = 0.05 and P = 0.01, respectively.

tion losses. Dithane D-14 and Vancide 51 are liquid fungicides and as such they probably did not reduce the firmness of the cellulose-fungicide pellet coat.

The three best treatments, pelleting with Orthocide 75, Dithane Z-78, and Tersan (Table 2), did not give significantly different results. Damping-off incidence with Tersan in the combined pellet and soil treatment was significantly higher than with Orthocide 75, Dithane Z-78, and Tersan seed treatments but lower than in the check. The combined treatment differed from the check also in the intensity and time of maximum mortality. This occurred suddenly and late, resulting partly, but not entirely, from phytotoxicity due to the accumulation of fungicide after six soil applications.

DISCUSSION

Soil treatments with acids may be beneficial in increasing the resistance of seedlings and changing favourably the soil flora (4). However, a serious objection to this standard method is the inconsistency in results (Table 1). A possible explanation of this is that damping-off in one nursery may be caused, as suggested above, by several pathogens which may differ in their tolerance to chemicals. Vaartaja* has demonstrated in a laboratory test that many chemicals, including sulphuric acid, are better tolerated by *Rhizoctonia solani* than by *Pythium debaryanum*.

Seed pelleting with certain new fungicides is worthy of further trials since the good results reported by Berbee *et al.* (1) were again encountered in the present study.

The fungicides that performed well (Table 2) are evidently low in their phytotoxicity to tree seeds and seedlings* (2). Satisfactory results have been reported for various treatments with these and related fungicides such as captan* (2, 9), zineb* (6), and thiram* (1, 2, 8, 9). However, the application of zineb in acid Michigan soil has given poor control (9). Ludwig and Thorn (6) have established that zineb may fail under acid conditions.

Unexpectedly the combined seed and soil treatment with Tersan was less effective than the seed treatment only. The germination in the combined treatment was good and seedling mortality was low at first, but finally reached higher totals than in the check (Table 2). In addition to some phytotoxicity, the cause is believed to be the severe disturbance of the microbiological balance and subsequent rapid reinfestation by *P. cactorum*, a "water mould" favoured by the rain.

Fungicides may depress saprophytes that would otherwise compete with and combat pathogens in soil. The natural biological control may thus be nullified by a fungicide. Probably this was the case in the combined treatment, but hardly in seed pelletings where the amount of Tersan was only moderate. Actually, moderate thiram applications on soil are known to have selective influence favouring certain useful saprophytes (7, 10). At least somewhere around the pellet there should be a zone where the fungicide concentration is such that this selectivity was exerted and thus a barricade against the pathogens was created. A test in Petri dishes has shown that besides thiram, captan and zineb are also selective so that *Rhizoctonia solani* is inhibited at a concentration where certain soil bacteria are still able to grow*. Consequently, all these fungicides may control damping-off not only directly but also indirectly.

Formaldehyde, though often injurious when applied 3 to 7 days before seeding (Table 1), may be a satisfactory fungicide if applied 1 to 2 months before sowing, as done by Warcup (12). Such early application partially sterilized the soil but in the interim the useful saprohytes that recolonized the soil brought about biological control against damping-off.

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SUPPLEMENTAL RIBOFLAVIN AND A FEED FLAVOUR IN CREEP FEED RATIONS FOR SWINE[1]

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ABSTRACT

Suckling pigs, offered a basal creep feed ration composed primarily of small grains with 10 per cent granulated white sucrose and containing an average of 1.5 gm. per ton of natural riboflavin, ate as much feed and made gains in the same range as pigs offered the same ration supplemented with 2 gm. of riboflavin, and/or 1 lb. of "Anifeed Dribase Pig Feed Flavor", per ton. A marked seasonal variation in both creep feed consumption and weaning weight was observed. It was calculated that 22 per cent of the variation in weaning weight of individual pigs was associated with creep feed consumption, irrespective of which ration was being consumed.

INTRODUCTION

Although there is a lack of precise information regarding the nutrient requirements of the pig prior to 8 weeks of age, it is generally acknowledged that under farm conditions creep feeding of suckling pigs is a worthwhile practice. A satisfactory creep feed should not only contain the necessary nutrients in the correct proportions but should also be highly palatable.

Riboflavin

There is a noticeable lack of agreement on the suggested riboflavin requirements of swine. This may be due to the fact that the riboflavin requirement of the pig varies with the climatic environment as demonstrated by Mitchell *et al.* (6). Although Forbes and Haines (2) and Miller *et al.* (5) have studied the riboflavin requirement of the baby pig, their results are probably of limited value in the formulation of creep feed rations as they maintained their experimental animals on synthetic diets. Using a practical type ration, Krider *et al.* (4) estimated that the weanling pig has a riboflavin requirement of 1.4 mg. per lb. of feed, which value is perhaps the most useful one available for the formulation of creep feed rations.

Feed Flavours

The use of flavour compounds to increase the palatability of feeds is a relatively recent development. The only available report of an experiment in which feed flavours were added to the rations of young pigs is that of Hanson *et al.* (3). To a series of basal creep feeds, "Flavor M. (molasses-fortifier)" and "Flavor A.M. (anise-molasses)" were added. On offering both basal and flavoured creep feeds free-choice to several groups of young pigs it was found that they consumed approximately twice as much basal as flavoured feed.

[1] Supported in part by a grant from the National Research Council of Canada.

[2] The data reported in this paper are a portion of a thesis presented by the senior author as partial fulfilment of the requirements for a Master of Science degree.

Investigations by Clandinin and Robblee* indicated that "Anifeed Dribase Poultry Feed Flavor" had no effect upon the feed consumption or rate of gain of chicks.

Due to this apparent lack of information on creep feed rations for swine, an experiment was conducted to determine whether the nutritive value and/or palatability of a creep feed ration, similar to that successfully used in previous years at this institution, could be improved by addition of supplemental riboflavin and a commercial feed flavour compound at levels of 2 gm. and 1 lb. per ton respectively.

MATERIALS AND METHODS

The farrowing of the purebred Yorkshire sows at the University of Alberta Livestock Farm during 1954 occurred in four definite periods, January, February-March, July and September. Prior to farrowing each sow was allotted at random to one of four groups for the purpose of conducting a creep feed experiment with the resulting litters. As the prepartum and lactation rations of sows may express an indirect effect upon the young pigs at weaning, these rations have been listed in Table 1. It should be noted that the sows farrowing in the summer had been on pasture until a few days prior to parturition, hence the absence of both alfalfa and vitamin A and D supplement in their prepartum rations.

The suckling pigs were weighed, as litters, at birth, 4 weeks of age, and at 54 days of age, the time of weaning. Sows were weighed at the same intervals, all weighings being executed on a beam-scale graduated at 0.5-lb. intervals. Feed was weighed on a spring-scale, graduated at 0.1-lb. intervals.

* *Personal communication*, 1955.

TABLE 1.—SOW RATIONS FOR CREEP FEED EXPERIMENT

	Pregnancy[1]		Lactation
	Spring[2]	Summer	
	lb.	lb.	lb.
Barley	600	600	500
Oats	1200	1200	1200
Wheat			200
Alfalfa meal (dehydrated)	200		
Concentrate (35 per cent crude protein)	200	200	200
Linseed oil meal			100
Wheat bran	100	100	100
Feeding oil[3]			3
Iodized salt	12	12	12
Ground limestone	12	12	12
Total	2324	2124	2327

[1] Spring relates to farrowings in January and February-March. Summer relates to farrowings in July and September.

[2] Also received an average of 1000 I.U. of vitamin A and 150 I.U. of vitamin D_3, administered as a feeding oil spread on top of the feed, once per day.

[3] Feeding oil contained 1000 I.U. vitamin A and 150 I.U. vitamin D_3 per ml.

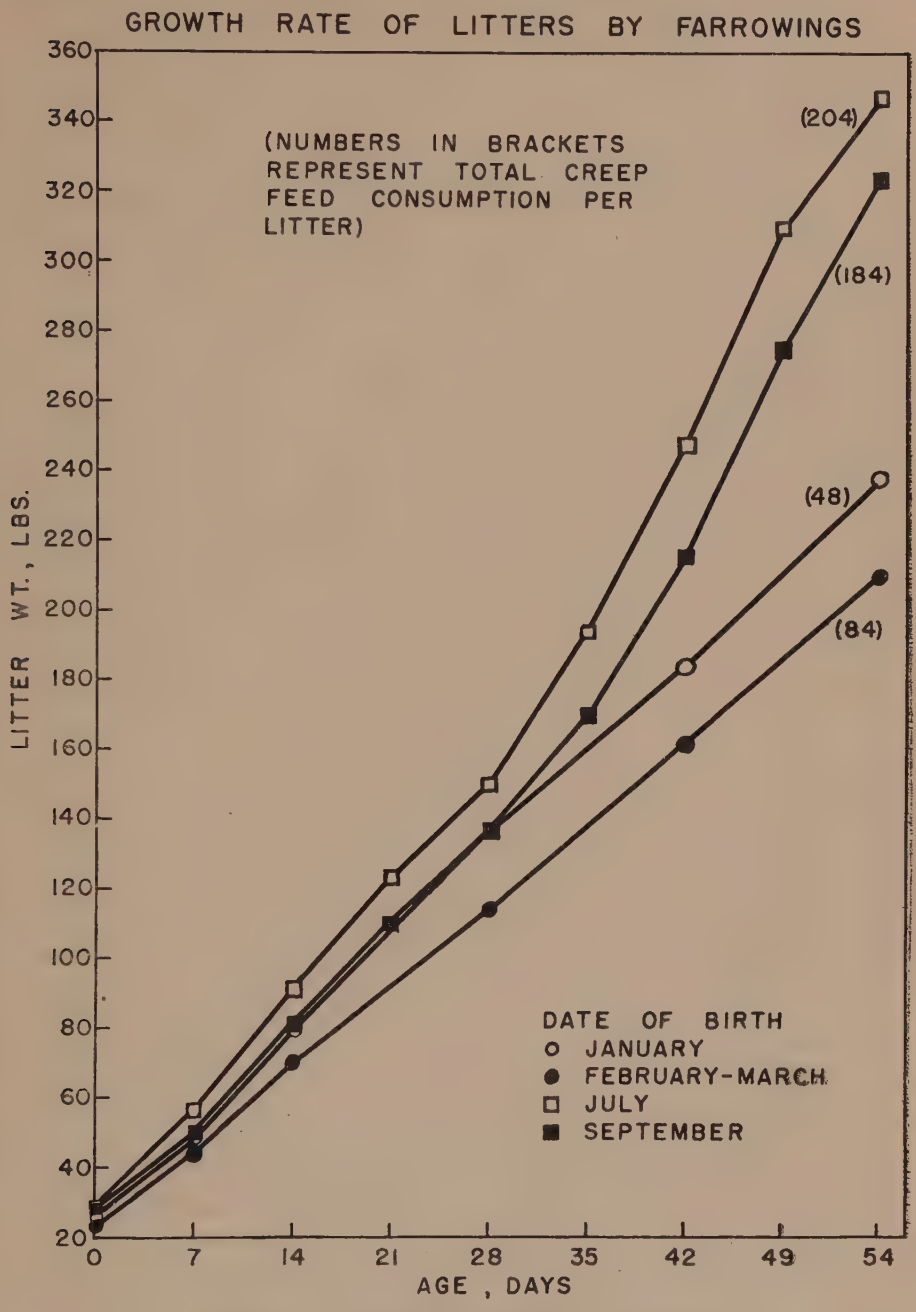

FIGURE 1. Growth rate of litters by farrowings.

When the young pigs were 2 weeks of age they were offered solid feed behind a creep in the corner of the pen. The creep feed ration offered was that appropriate to the group to which the litter had been allotted prior to birth. The four creep feed rations were as listed in Table 2.

TABLE 2.—COMPOSITION OF CREEP FEED RATIONS

1. Basal

Wheat (coarsely ground)	55 lb.
Oat groats (coarsely ground)	22 lb.
Meat scraps	4 lb.
Soybean oil meal	7 lb.
Sugar (granulated white sucrose)	10 lb.
Vitamin B_{12}—Aureomycin supplement[1]	1 lb.
Iodized salt	0.5 lb.
Ground limestone	0.5 lb.

Dry "A" and Dry "D" to supply 100,000 I.U. of vitamin A and 20,000 I.U. of vitamins D_2 per 100 lb.

2. Basal ration + 2 gm. riboflavin/ton
3. Basal ration + 1 lb. of feed flavour[2]/ton
4. Basal ration + 2 gm. of riboflavin + 1 lb. feed flavour/ton

[1] Lederle "Aurofac" containing 1.8 mg. of vitamin B_{12} and 1.8 gm. of Aureomycin hydrochloride (chlortetracycline) per pound.

[2] "Anifeed Dribase Pig Feed Flavor" containing extractives of anise, cinnamon, clove, birch, orris, butryic acid, vanillin, soya meal and corn sugar. Supplied through the courtesy of N. D. Hogg Ltd., Toronto, Ont.

Analysis of the various mixes of the creep feed rations indicated that the protein content was approximately 16 per cent, the range being from 15.7 to 16.7 per cent. Crude fibre content of the rations never exceeded 3 per cent, the range being 2.5 to 2.9 per cent. The riboflavin content of the basal creep feed ration, determined fluorometrically by the method of the Association of Vitamin Chemists (1), was found to be in the range of 0.65 to 0.80 mg. per lb., with a mean value of 0.75 mg. per lb. of feed.

RESULTS

A summary of the data obtained from the experiment previously described is given in Tables 3, 4 and 5. The growth rate of litters by farrowing periods is expressed graphically in Figure 1, while the growth

TABLE 3.—AVERAGE LITTER WEIGHTS AND RANGE OF WEIGHTS AT BIRTH, 28 AND 54 DAYS OF AGE

	No. of litters	Birth		28 days		54 days	
		Mean	Range	Mean	Range	Mean	Range
		lb.	lb.	lb.	lb.	lb.	lb.
Date of Farrowing							
January	10	25.3	16.0–30.1	127	94–171	232	178–304
Feb.-March	12	23.1	15.1–34.2	114	68–142	209	122–277
July	12	28.9	15.0–38.0	154	109–219	353	216–463
September	15	28.0	17.7–39.0	136	86–171	320	180–419
Ration							
Basal	12	25.4	15.1–33.0	128	68–171	274	122–419
+ riboflavin	13	24.5	15.0–32.0	133	86–167	266	180–364
+ flavour	12	28.6	21.1–39.0	145	123–177	307	178–397
+ riboflavin and flavour	12	27.0	16.0–38.0	162	92–219	287	179–463
Average; all effects	49	26.5	15.0–39.0	135	68–219	283	122–463
L.S.D.		4.8				47	

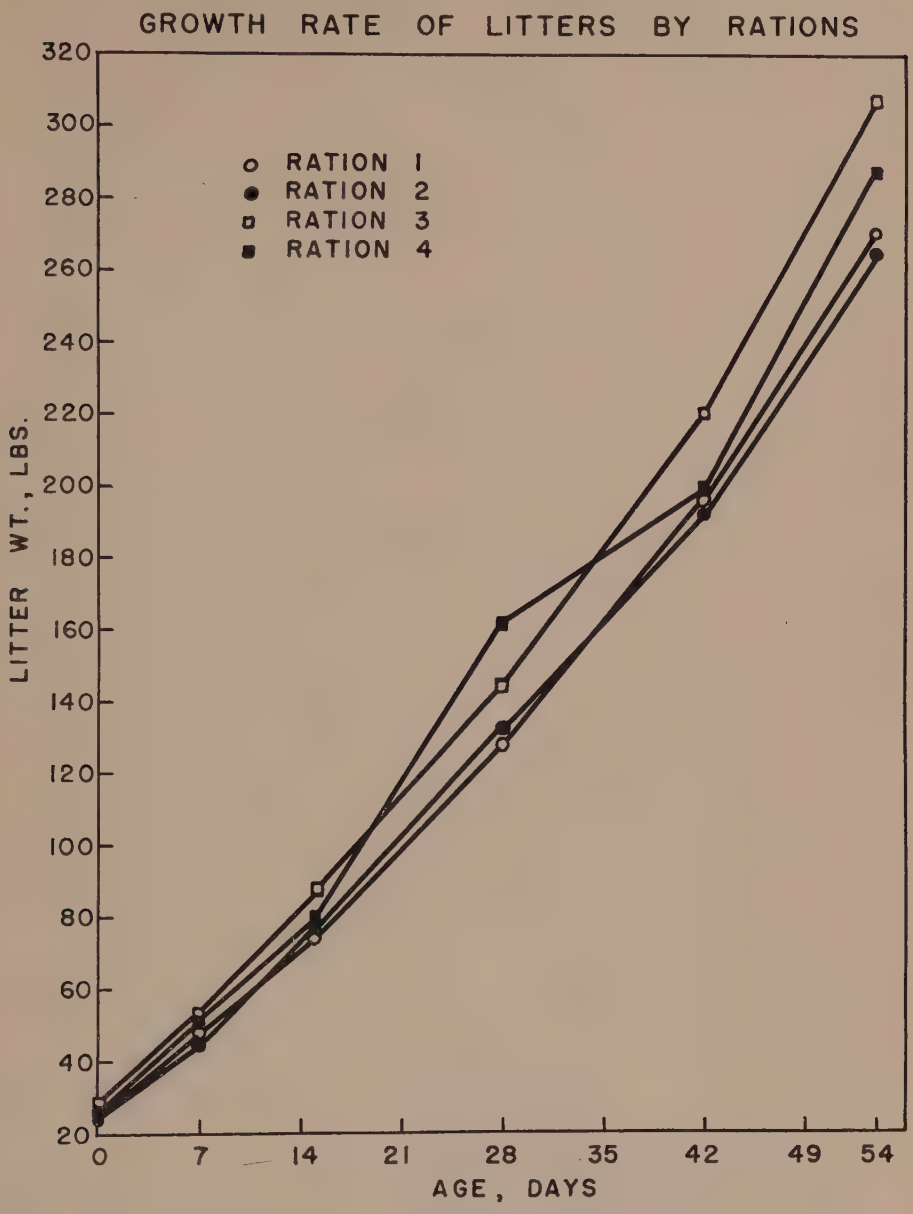

FIGURE 2. Growth rate of litters by rations.

rates of the different ration groups, irrespective of date of birth, are indicated in a similar manner in Figure 2. The data presented in Tables 3, 4 and 5 are difficult to interpret, as a great many factors have a potential influence on the weaning weight of a pig, or of a litter. A few of these variables are: birth weight, amount of milk received (this is probably influenced by both feed consumption and loss in weight of the sow), number and sex of pigs, creep feed consumption, and several other minor factors.

TABLE 4.—FACTORS HAVING A POTENTIAL INFLUENCE ON BIRTH WEIGHT AND WEANING WEIGHT

	Loss in weight of sows[1]		Feed consumed by sow		Creep feed consumed per litter	
	Mean	Range	Mean	Range	Mean[2]	Range
	lb.	lb.	lb.	lb.	lb.	lb.
Date of Farrowing						
January	90	22–175	735	572–818	47	14–107
Feb.-March	68	23–152	766	624–910	79	15–205
July	69	27–101	891	691–963	204	15–315
September	76	23–147	859	821–890	200	27–340
Ration						
1.	72	25–122	811	624–951	114	15–265
2.	83	23–175	821	647–963	119	20–340
3.	81	22–175	836	710–930	150	14–267
4.	64	22–104	809	572–917	159	14–315
Average, all effects	75	22–175	819	572–963	135	14–340

[1] Loss in weight from (approximately) 8 hours prior to farrowing until time of weaning.
[2] L.S.D. for creep feed consumption = 62 lb.

TABLE 5.—AVERAGE PIG WEIGHTS AND CREEP FEED CONSUMPTION

	Age of pigs			Creep feed consumed per pig
	Birth	28 days	54 days	
	lb.	lb.	lb.	lb.
Date of Farrowing				
January	2.6	14.3	25.2	5.1
Feb.-March	2.9	13.4	24.8	9.3
July	2.8	16.4	37.2	21.9
September	2.6	14.2	33.6	18.0
Ration				
Basal	2.6	14.4	30.4	12.6
+ riboflavin	2.7	15.6	32.2	14.7
+ flavour	2.6	13.7	29.2	14.3
+ riboflavin and flavour	2.8	14.8	32.2	17.8
Average, all effects	2.64[1]	14.6	30.8[2]	14.7
L.S.D.	0.31		3.6	6.4

[1] 492 pigs born averaging 10.04 pigs per litter.
[2] 450 pigs weaned averaging 9.18 pigs per litter.

TABLE 6.—MEAN SQUARES, OBSERVED AND NECESSARY F VALUES FOR SIGNIFICANCE IN BIRTH WEIGHTS, WEANING WEIGHTS AND CREEP FEED CONSUMPTION

Trait studied	Between rations		Between seasons		Necessary F values	
	M.S.	F	M.S.	F	0.05	0.01
Birth weight						
by litters	.48	1.41			2.82	4.26
by average pigs	0.057	0.39			2.82	4.26
Weaning weight						
by litters	3,350	1.04	55,840	17.31	2.90	4.46
by average pigs	22.19	1.13	479.91	24.47	2.90	4.46
Creep feed intake						
by litters	5,138	0.91	68,919	12.26	2.90	4.46
by average pigs	67.16	1.14	631.38	10.76	2.90	4.46

A statistical analysis of the data has been conducted and is summarized in Table 6. For simplicity of calculation and to maintain orthogonal design, one sow and litter were eliminated at random from Lot 2 (basal + 2 gm. riboflavin/ton) prior to analysis. This left 12 litters per creep feed ration group. It is evident from the data in Table 4 that allotment within ration groups was reasonably uniform in so far as loss in weight and feed consumption of the sows were concerned. Seasonal differences which may occur in these variables were not considered in this study as they were removed in the subsequent statistical analysis.

Although the sows were selected at random, it was decided to carry out a preliminary analysis of variance of the total birth weight of the litters and also of the average birth weight of the pigs, between ration groups, to determine whether randomization had been successful in regard to these measures.

This analysis would indicate that the selection of sows was random as the differences in birth weights of litters or average pigs between lots were not likely to influence subsequent gains or creep feed consumption.

A further analysis was conducted to determine the effect of rations on weaning weight and creep feed consumption with any seasonal effects removed. As with the data relating to birth weight this analysis was first conducted on a litter weight basis, and second on an average pig basis.

By analysing these data on the basis of average pig values, it was hoped that any non-random variation due to the number of pigs in the litters would be removed. Examination of the analysis indicates that the results obtained when average pig data were used are very similar to those obtained when litter values were utilized.

The analysis in Table 6 of the data summarized in Tables 3, 4 and 5 indicates that there was no significant difference between the weaning weights or creep feed consumption of the different ration groups. There was, however, a highly significant seasonal difference in both weaning weights and creep feed consumption.

A correlation between weaning weight and creep feed consumption with seasonal variations, and the variations due to the number of pigs in the litter removed yielded an r value of 0.465 which is significant at the 1 per cent level. This r value (40 D.F.) indicates that approximately only 22 per cent of the variation in average pig weaning weights within periods is associated with creep feed consumption.

DISCUSSION AND CONCLUSIONS

The analyses of variance would indicate that there was no significant difference between the four ration groups respecting birth weight, weaning weight or creep feed consumption. These would indicate that, under the particular conditions of this trial, "Anifeed Dribase Pig Feed Flavor" did not increase the palatability of the creep feed ration above a level which could be explained by chance fluctuations. These results bear a similarity to those of Hanson *et al.* (3), where an anise-molasses mixture did not increase the palatability of a creep feed ration. It is still quite possible that feed flavours may be of value in creep feed rations but this trial would indicate that the particular flavour used had no demonstrable beneficial effect.

That the nutritive value of the basal creep feed ration did not appear to be increased by the addition of 2 gm. of riboflavin per ton would indicate that the content of the basal ration (1.5 gm. per ton by analysis) was sufficient for the needs of the suckling pigs. It is difficult to compare this value with those previously given in the introduction as the suckling pigs would also receive an unmeasured quantity of riboflavin from the sows' milk. However, it is felt that the value of 1.5 gm. per ton or 0.75 mgm. per lb. of feed is not very different from the requirements of the pig as already listed. It seems that the riboflavin content of the basal creep feed ration did not limit the growth rate of suckling pigs and that creep feed formulated on a basis of wheat and oat groats would be unlikely to require a riboflavin supplement.

ACKNOWLEDGEMENTS

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LEAF FEEDING OF DETERMINATE TOMATO PLANTS
1. THE INFLUENCES OF ENVIRONMENT[1]

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ABSTRACT

Water solutions of urea and sucrose at 0.5 M. concentrations, alone and combined, were used as foliar sprays on determinate tomato plants in greenhouse trials. Leaves absorbed and utilized urea applied in spray form but fruit maturity was retarded and the C/N ratio in leaves was depressed in comparison to that observed in root-fed plants. No direct evidence was obtained of sucrose absorption, but the addition of an equi-molar concentration of sucrose brought about a marked reduction in foliar injury due to urea spray. This combined spray significantly increased the development of plant tops and resulted also in abnormally large accretion of starch in plant stems, and soluble carbon in the fruits. On the other hand, combining sucrose with urea resulted in certain unfavourable responses: Fruit ripening was delayed, fruit set was normal, but the proportion was low of fruits that developed in size beyond the early post-setting stage, and total yield of fruit was reduced.

INTRODUCTION AND LITERATURE REVIEW

When fertilizers are applied broadcast or by hand applications at planting time, heavy losses often occur through leaching, surface run-off and the fixing of nutrients into insoluble compounds. This has led certain investigators to study the feasibility of supplying nutrients to the foliage of plants, as they are required. The leaf-feeding of horticultural crops recently has been reported by Cornell (1) and Wisconsin (6) workers, and by Emmert and Klinker (4) for indeterminate tomato plants under Kentucky conditions. The rather stringent environment imposed upon the development of tomato plants under Canadian prairie conditions, the virtual dependence of the area upon determinate varieties, and the critical nature of soil fertility problems in the short time available between blossom truss formation and fruit maturity, make the study of nitrogen metabolism in relation to environmental factors of particular interest to research workers and growers alike.

Under field conditions at Edmonton, the authors have observed that urea application to Early Alberta tomato foliage resulted in definite increases in soluble leaf nitrogen in 1 to 2 days after spraying, and that the level returned to normal after 8 to 12 days. Sucrose applied in equimolar combination with urea prolonged the absorption period and practically eliminated the burning effect evident where urea sprays were applied alone. A report on this work is in process of preparation. Emmert and Klinker (4) showed, as did field trials at Edmonton, that the leaf burn became evident about 24 hours after the internal level of soluble nitrogen reached its highest point. This burning effect is believed to be due to toxic accumulations of ammonia as a result of rapid enzymatic hydrolysis of absorbed urea.

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Cook and Boynton (1), in applying urea to leaves of McIntosh apple trees, found the rate of absorption to be rapid under ideal growing conditions, and widely variable at other times. The use of wetting agents increased the rate of absorption, and a decrease in vapour pressure gradient between spray droplets and the atmosphere also positively affected the intake of urea solution. Went and Carter (8) held tomato plants in darkness after spraying them with sucrose, and a growth response was noted within 6 days. Smith and Zink (7) have reported that absorption and utilization of sucrose sprayed on young tomato plants occur within 4 days of spraying, and evidence of sucrose intake 2 days after spraying appears to have been obtained by Emmert and Klinker.

The rate of photosynthetic activity is positively correlated, within certain limits, with increases in temperature. However, as high temperatures and vapour pressure deficits coincide with higher rates of transpiration and a subsequent deficiency of internal water, the rate of photosynthesis will be reduced. Therefore, within the temperature range of 10° to 35° C., that is associated with the active conversion of solar energy, the net accumulation of carbohydrates may be less at high than at low temperatures. The interrelationship of these factors with nutrient intake and utilization led to a study of the effect of environment upon the use made by tomato plants of nitrogen applied to leaves.

MATERIALS AND PROCEDURE

Greenhouse Trials

During the early winter of 1951 and early in 1952, a preliminary trial was carried out in which sprays of 0.5 M. concentrations of urea and sucrose alone and combined were carefully applied to the foliage of Early Alberta tomato plants. These plants were well-established in 6-inch clay pots containing composted clay loam, soil and sand (3:1 by volume), and were 15 to 18 inches high with a single cluster of flower buds at the time of treatment. Two plants were left unsprayed as a duplicate control treatment. The weather was dull at the time of treatment and this condition prevailed generally throughout the trial. As a result, the plant leaves, though normal green in colour, were thinner than usual for tomato plants grown under more intense light and longer photoperiod.

Leaf samples were taken daily from December 27 to January 3, and soluble nitrogen and carbon estimations were carried out by the methods outlined by Emmert and Klinker (4), although these were slightly modified to suit conditions of this trial. A leaf extract was prepared from a 1-gm. sample of fresh leaf and macerated with a gram of charcoal and 5 ml. of 2 per cent acetic acid. A further 5 ml. of the acetic acid solution was added during grinding and the extract was filtered. Analyses were carried out as quickly as possible after extraction. Nitrogen estimation involved the use of 0.5 ml. leaf extract treated with a small excess of $NaClO_3$ (approximately 0.05 gm.) and adding 1 ml. fuming H_2SO_4. After cooling, 1 ml. phenoldisulphonic acid was added, the solution agitated, 12-15 ml. distilled H_2O added, and NaOH until the solution was basic. The yellow solution was diluted to 30 ml. with distilled H_2O and a photometric comparison was made with a similarly prepared blank. Conversion to p.p.m.

sol. N was made by means of a prepared calibration curve. This test is assumed to estimate nitrogen of nitrate, amino compounds and urea, and proved to be rapid and adequate for the purpose of the work reported here.

Soluble carbon estimation utilized 1 ml. leaf extract in a 30-ml. test tube calibrated at the 25-ml. level. Two ml. fuming H_2SO_4 were added with agitation. After 10–15 min., the solution was made up to 25 ml. with 50 per cent (by volume) C.P. H_2SO_4. The coloured fraction was distributed evenly by gentle agitation, allowed to stand for 4 hours, and the photometric determination carried out in conjunction with a blank. Soluble carbon as p.p.m. was estimated from a calibration curve established earlier. This test estimates total soluble carbohydrates, but includes such soluble carbon as may be present in soluble amino or organic acids.

Each leaf was carefully washed in tepid distilled water and dried prior to extraction procedure. On the evening of January 2, three 300-w. G.E. self-reflector lamps were placed 12 inches above the plant apices and the plants were illuminated for 21 hours until the final leaf samples were taken on January 3.

On May 30, 1952, 24 young Early Alberta tomato plants were washed clean of soil, weighed and transplanted into 9-inch pots containing washed sand. The pots were arranged in shallow watering trays in a 6 × 4 grid, spaced at 1 foot apart. After a 2-week period,during which all plants received a complete Ellis and Swaney (3) nutrient solution, it was noted that all plants were mildly chlorotic. A check on nutrient levels by Spurway quick-test technique indicated high phosphorus and inadequate nitrogen. The Davis and Hill (2) method of feeding tomatoes then was used, supplemented with additional ammonium nitrate, and by July 14 when differential treatments were begun, all plants were vigorous, uniform and normal in appearance. From that time until July 29, treatments were as follows:

1. *Nitrate*—50 ml. nutrient solution containing 2.33 mg. N applied to roots daily.
2. *Urea*—Mode of application and amount of N as in (1).
3. *Urea*—Application containing 16.81 mg. N in water spray to foliage weekly. 50 ml. N-free nutrient solution to roots daily.
4. *Urea and Sucrose*—as in (3), but with sucrose added to spray solution in equi-molar rate.

Since the principal objective in this trial was to compare the effects of root and leaf applications of urea, and the control treatment was that in which nitrate nitrogen was applied to the soil, a sucrose alone treatment was not included.

From July 29 to September 3, the rate of feeding of all nutrients was doubled, to compensate for increased plant requirement. All pots were leached once weekly with 2 to 3 quarts of water, and before sprays were applied a slotted masonite disk was placed over each pot to minimize entry of spray drip into the sand. Spraying was done with a small hand sprayer capable of delivering all of the liquid (7 ml.) necessary for each plant. It was estimated that 80–85 per cent of the spray reached the leaves and stem and was retained there.

Vegetative development of the plants was rather weak, and in order to maintain the photosynthetic function to the fullest degree until a fruit harvest was made, a single set only of leaf samples was taken (August 13) for soluble carbon and nitrogen analyses. The first harvest of fruits was

recorded on August 26. The remaining fruits were picked and weighed on September 3, at which time representative samples of fruits were analysed. Fresh and oven-dry weights were recorded for all parts.

Because of the difficulty in stabilizing the development of the tomato plants in the second trial, a similar set of plants was transplanted to pots of leached sand on September 17, 1952. These were fed a complete nutrient solution for 30 days and treatments were begun on October 17, by which time one or two fruits had set on the first flower cluster on all plants. Treatments were as before, except that the rate of nitrogen feeding was increased from 2.33 mg. to 3.50 mg. daily. The feeding rate was kept constant until the trial ended on January 6, 1953. Records additional to those taken earlier were: Number of fruits per replicate; and numbers of fruits that set but failed to size properly. In addition, refractometer readings were taken on juice expressed from ripe and green fruits, ripe fruits were rated organoleptically and micro-chemical starch tests (KI and I_2 solution) were made on middle stem sections of plants in all treatments.

Growth Chamber Trials

During January to March, 1953, growth chamber tests were arranged to determine the influence of high temperature upon 8- to 10-inch Early Alberta tomato plants that were treated with 0.5 M. urea sprays. Light intensities of 125, 350 and 650 foot-candles were supplied by fluorescent units, temperature was maintained at 24 $\pm$ 3° C., and two conditions of relative humidity were set up. These were: (1) constant RH 88 $\pm$ 2 per cent; and (2) variable RH 45 $\pm$ 7 per cent during day and RH 60 $\pm$ 10 per cent at night. Each lot of plants received a photoperiod of 8 hours.

Two other sets of plants were sprayed with 0.5 M. urea and placed in a low temperature chamber at 10 $\pm$ 2° C. One set received a light intensity of about 150 foot-candles, the other 350 to 400 foot-candles and the photoperiod also was 8 hours. Control of relative humidity was not possible and the variation was RH 40 to 60 per cent during days and 80 to 90 per cent at night.

TABLE 1.—SOLUBLE NITROGEN LEVELS IN TOMATO LEAVES IN WINTER GREENHOUSE TRIAL. (AS P.P.M. OF FRESH LEAF; MEANS OF DUPLICATE SAMPLES)

Days after spraying	Control	0.5 M. Urea	0.5 M. Sucrose	0.5 M. Urea + Sucrose
1	1848	2409	1287	1661
2	1501	2964	1617	1199
3	1276	2706	1001	1315
4	1012	1612	1188	742
5	1859	1969	1766	1210
6	1458	1573	1100	1254
7	979	1194	1188	935
8*	792	847	924	979
Means	1341	1909	1258	1168

* Plants illuminated and subjected to higher temperature throughout night of 7th day.
Difference necessary for significance between treatments (P = .01) = 486 p.p.m.

RESULTS AND DISCUSSION

The winter greenhouse trial provided information on the soluble-nitrogen variation in treated plants over a period of 8 days following leaf-spraying. This is summarized in Table 1, from which it will be observed that while the soluble nitrogen content fluctuated considerably within treatments, the plants sprayed with urea alone showed significantly higher amounts than those otherwise treated. The result of the combination of urea and sucrose, on the other hand, strongly suggests that the latter compound inhibits early absorption of urea.

Soluble carbon levels were negative or "trace" throughout the first 7 days after spraying, but after the period of illumination the analyses on the eighth day gave the following readings of soluble carbon as p.p.m. of fresh leaf tissue: *Control*—1100; *Urea* (0.5 M.)—1750; *Sucrose* (0.5 M.)—1435; *Urea* + *Sucrose* (0.5 M. each)—758. The single day's results did not allow statistical interpretation, but it would appear that the early absorption of urea nitrogen as indicated in Table 1 produced a condition allowing very rapid synthesis of carbohydrates when adequate light was provided.

The urea sprays did not cause any leaf-burn for the first 7 days after application, but within 15 hours following the incandescent illumination of the plants, slight burning was evident on plant leaves that received urea alone. Later on the eighth day, the burning was more severe, affecting two-thirds of the upper leaves and up to 10 per cent of individual leaf surfaces. An observed temperature gradient of 8° to 10° C. between top and base of plant would seem to be responsible for the more severe injury on apical leaves. Barely noticeable burning was present on the eighth day on plants sprayed with the combined urea + sucrose treatment, and no leaves on the two unsprayed plants showed any burning.

TABLE 2.—SOLUBLE CARBON IN TOMATO LEAVES AND RIPE FRUITS IN SUMMER GREENHOUSE TRIAL (AS P.P.M. OF FRESH TISSUE; MEANS OF SIX REPLICATES)

	Fed via Roots		Fed via Leaves	
	NO_3	Urea	Urea	Urea + Sucrose
Leaves (Aug. 13)	1162	1035	1081	1414
Fruits (Sept. 3)	7421	5779	8176	10309

TABLE 3.—TOMATO FRIUT YIELDS IN SUMMER GREENHOUSE TRIAL

(In gm.; means of six plants)

	Fed via Roots		Fed via Leaves	
	NO_3	Urea	Urea	Urea + Sucrose
Early Ripe (Aug. 26)	121	174	147	150
Final Ripe (Sept. 3)	150	80	130	114
Total Green (Sept. 3)	42	62	42	36
Totals	313	316	319	300

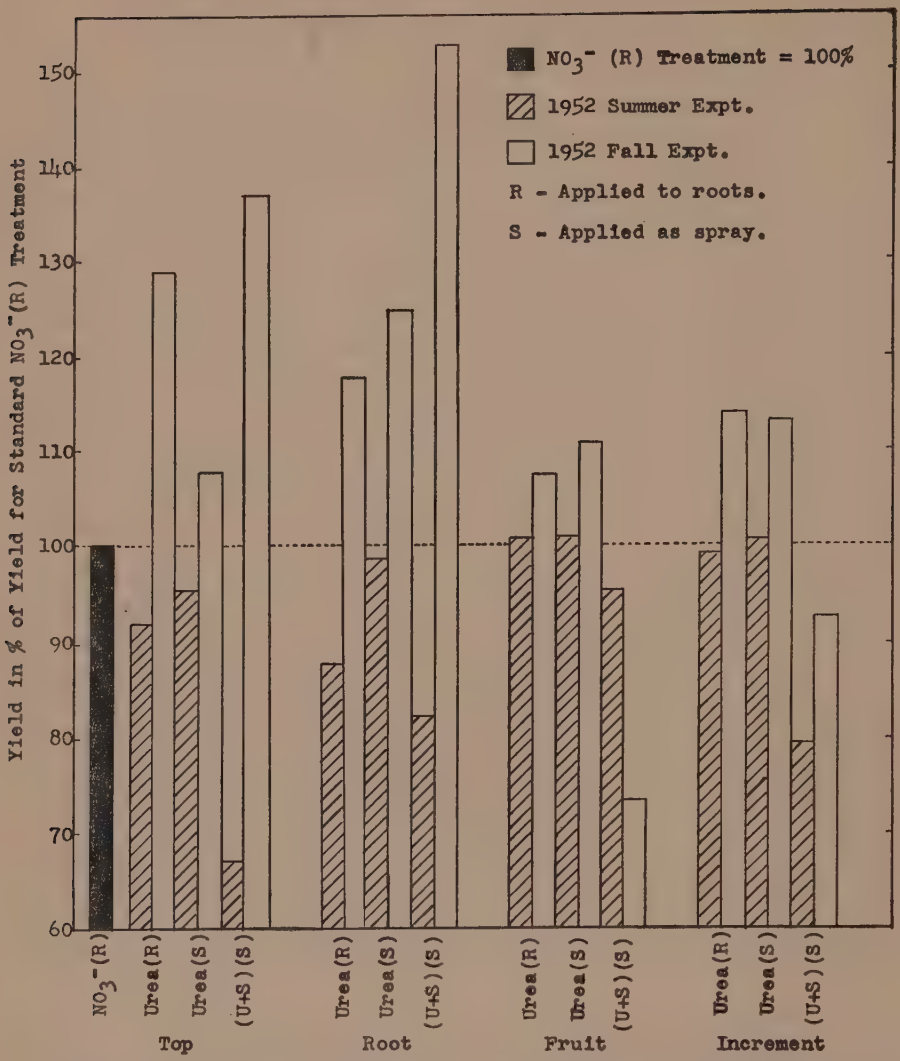

FIGURE 1. Histographic comparison of yields of plant parts, plus increment in fresh weight during the experimental periods for summer and fall greenhouse experiments. Values for the nitrate-fed plants are taken as 100 per cent in all instances.

Table 2 is a summary of the soluble carbon data for leaves and fruits from the summer greenhouse trial. The differences noted are not of a significant nature, and determinations of soluble nitrogen showed a "negative" reading, corresponding to less than 10 p.p.m., fresh weight basis. Vegetative growth of these plants was weak but none of the plants showed visible symptoms of nitrogen deficiency after the rate of fertilizing was increased. Fruit yield is shown in Table 3 and again the differences present are not statistically significant.

Figure 1 provides a comparison of top, root and fruit development for the two greenhouse trials. The fresh weight of roots and dry matter of tops from the combined urea and sucrose treatment were significantly greater than those from other treatments.

TABLE 4.—NITROGEN FERTILIZING INFLUENCES ON VARIOUS TOMATO FRUITING CHARACTERISTICS IN AUTUMN GREENHOUSE TRIAL

	Fed via Roots		Fed via Leaves	
	NO_3	Urea	Urea	Urea + Sucrose
No. fruits set	50	71	69	59
No. fruits sized	11	17	19	19
% fruits sized	22	24	27.5	32
Mean weight of sized fruit (gm.)	50.9	35.4	34.4	21.5
Mean content sol. carbon*	4939	6273	6430	7907
% total sol. solids (ripe fruit)	3.8	5.3	4.5	5.3

* As p.p.m. of fresh weight (ripe fruits).

Table 4 summarizes records taken from plants in the autumn greenhouse trial. The data show that urea, whether root-fed or sprayed on the plant leaves, was associated with a depression in fruit size, with a further size decrease in plots receiving the combined spray. The soluble carbon content increased as the final size of the fruits decreased. Refractometer readings show that in general the ripe fruits from treatments receiving urea were highest in total soluble solids. In taste panel tests, no obvious differences were noted except in fruit from the urea plus sucrose treatment. This was unanimously classed as definitely sweetest, although the total solids content does not differ from that found in fruits from plants receiving urea through their roots.

Went and Carter (8) and Emmert and Klinker (4) have suggested that the reduced light conditions of winter are those most likely to show a favourable effect of sucrose sprays on general tomato plant development. Figure 1 indicates that this effect is true in the present work only for root and top increments, but that when the yield of fruit is taken into account there is a reduction in total development in the treatments involving sucrose. This applies to both the summer and the autumn tests, but the depression in fruit development is particularly marked in the latter. The increases in the yield of tops of plants during the autumn trial are highly significant where plants receive urea via roots and urea and sucrose combined as a spray; in root weight all urea treatments are significantly more effective than is the case with control plants treated with root-applied nitrate. Urea sprays, alone and in combination with sucrose, are observed to retard fruit sizing and ripening.

Examinations of the stems of sand-cultured plants showed that heavy starch accumulation was present in all tissues adjacent to vascular bundles in plants receiving the urea plus sucrose spray, but not in root-fed plants nor in plants receiving urea spray alone.

Growth Chamber Trials

At 90 per cent relative humidity, the urea "burn" decreased as the light intensity increased. When the humidity was decreased rapidly after two 8-hour photoperiods in 48 hours, the burn effect became very marked. This apparent increase in "burn" as a result of lowered humidity presumably was due to the faster desiccation of the injured tissues under this condition. At 125 f.c., an estimated 70 to 90 per cent of the leaf area was affected; at 350 f.c., about 50 to 60 per cent; and at 650 f.c., about 40 to 50 per cent. After a further 3-day period, at reduced humidity, plants receiving most light were the only survivors.

Under variable conditions of humidity (45 per cent by day and 60 per cent by night), a similar gradient of leaf injury due to light intensity was observed. Two 8-hour photoperiods in 48 hours resulted in 15 to 25 per cent leaf area burned at 125 f.c., 12 to 20 per cent at 350 f.c., and 5 to 8 per cent at 650 f.c. When the same plants were exposed to 90 per cent humidity for 24 hours, followed by 24 hours at low humidity, some further injury was noted which was about the same for all light levels.

When low growing temperatures (10° C.) were imposed on plants, 8 hours at 150 f.c. and at 350 to 400 f.c. in a 24-hour period showed no urea spray injury on any plants. A second photoperiod resulted in slight leaf-tip injury, and this was most evident on plants receiving least light. The damage spread during the next 16 hours, but the differences due to light intensity remained stable.

CONCLUSIONS

Leaves of Early Alberta tomato plants proved capable of absorbing and utilizing nitrogen fed as urea by foliar sprays, but in comparison with supplying nitrogen to the plant roots, the spray treatment retarded fruit ripening slightly. The addition of sucrose to the urea spray solution reduced the level of leaf nitrogen significantly compared to that determined in leaves sprayed with urea alone, and the combined spray also inhibited fruit development. It is assumed that this effect is due to the sucrose acting in some manner to prevent the absorption of urea, and this in turn would result in a nitrogen lack for protein synthesis. Where reasonably adequate nitrogen was available in the growing medium, as has been observed in field trials at the University of Alberta, there was no effect on fruit maturity of the urea plus sucrose spray.

The leaf-burn usually associated with urea spraying is thought to be a result of toxic ammonia concentrations, derived within the leaf through rapid enzymatic hydrolysis of absorbed urea. Sucrose added to the urea spray almost totally eliminates the leaf injury, but in sand cultures under greenhouse conditions, 0.5 M. concentrations of urea and sucrose produced none of the symptoms of abnormal C/N relationship that if present would argue against leaf absorption of urea. In field trials at Edmonton, the authors found that the C/N relationship in tomato leaves was altered by spray treatments with urea and urea plus sucrose. Moreover, in the present trial a urea source of nitrogen, root-fed or leaf-fed, was associated with greater vegetative development and higher soluble carbon content in

ripe fruits, when compared with analyses on samples taken from nitrate-fed plants. These findings suggest that, in fruits produced under greenhouse culture, some alteration of the carbohydrate-nitrogen balance is effected by the use of urea nitrogen, and that this effect is expanded when sucrose is added to the spray.

Under conditions of low temperature, high light intensity and low relative humidity, a steady and relatively rapid rate of urea absorption through tomato leaves was noted, and this relationship is least likely to cause the leaf injury usually associated with urea sprays of concentrations above 0.1 M. Light and temperature are the most important climatic factors involved, and it would appear that urea may be most safely used as a foliage spray during periods of prolonged bright sunlight and relatively low temperatures.

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NON-IONIC SURFACTANTS IN CONCENTRATE MIXTURES FOR THE CONTROL OF APPLE SCAB[1]

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ABSTRACT

The addition of two non-ionic surfactants to concentrate fungicidal spray mixtures for the control of apple scab improved the effectiveness of the sprays investigated. The addition of either surfactant increased the amount of spray injury from fungicides which tended to be phytotoxic.

In high volume spraying it has been shown repeatedly that the effectiveness of direct contact insecticides such as nicotine sulphate can be increased by the addition of an appropriate surfactant to the spray mixture (5). The addition of a surfactant to a preventive spray may, however, reduce spray retention (1, 3) because of increased "run-off" of spray liquid. In concentrate spraying, on the other hand, the amount of spray liquid applied is considerably less than that required to produce the "run-off" characteristic of high volume application. Therefore the addition of a surfactant to a concentrate spray mixture should result in increased spray retention (4) and more uniform coverage of the fruit and foliage. Experiments in progress in British Columbia since 1951 indicate that the addition of an appropriate non-ionic surfactant increases the insecticidal effectiveness of DDT concentrate spray mixtures. The experiment reported here was undertaken in 1954 to investigate the effect of surfactants on fungicidal concentrate spray mixtures for the control of apple scab.

EXPERIMENTAL

Orchards

The experiment was conducted at Sunshine Bay, on the west arm of Kootenay Lake, where apple scab infection is consistently more severe than in any of the other fruit-growing areas of the interior of British Columbia. One orchard of 35-year-old trees (Sewell) contained 59 McIntosh and 40 Northern Spy trees which, in 30-foot square planting, ranged in height from 18 to 20 feet and had a spread of 25 to 30 feet. They were of average vigour, and pruned fairly well, although somewhat more bushy than the average trees in British Columbia. The fairly heavy crop was not thinned. In another orchard (Fransen) of the same age, about a mile distant, there were 13 Red Delicious, 9 Rome Beauty, 22 McIntosh and 28 Northern Spy. The trees were similar in size to those in the Sewell orchard, but had denser foliage and carried a moderate, reasonably well-thinned crop.

Seven plots were laid out in each of the two orchards, one plot serving as a check. Each plot contained sufficient McIntosh trees for comparable records of scab infection. The second orchard served as a replicate of the treatments applied to the first orchard. It was impractical to replicate within orchards since plot size would have been too small for experimental spraying with the equipment used.

[1] Contribution from the British Columbia Department of Agriculture, Victoria, B.C., and the Chemistry Division, Science Service, Canada Department of Agriculture, Ottawa.

[2] District Horticulturist, Creston, B.C.
[3] Chemistry Division, Summerland, B.C.

Weather

Cool weather prevailed throughout most of the growing season in 1954, particularly in May and June. While temperatures were below normal, precipitation was above normal. Records show that there was 3.40 inches of rainfall in May; 3.79 inches in June; 1.59 inches in July, and 4.37 inches in August. Much of the rain fell in prolonged light showers.

Equipment and Materials

Spray chemicals were applied by a 1954 model "Turbo-mist"* concentrate sprayer, operating pressure was 300 p.s.i. and the air velocity at the "fishtail" air vent was 110 m.p.h. The sprayer was equipped with a three-nozzle manifold on which the nozzle orifices for the first five sprays

*Okanagan Turbo Sprayers Ltd., Penticton, British Columbia.

TABLE 1.—SPRAY CHEMICALS, DOSAGES AND NUMBER OF APPLICATIONS

Plot No.	Materials applied	Rate per acre	Stage of application
1	Lime sulphur	8 gal.	Pre-pink, pink and full bloom
	Ferbam, 76% wettable powder	5 lb.	4 cover sprays
	Wettable sulphur	15 lb.	
2	Lime sulphur	8 gal.	Pre-pink, pink and full bloom
	Triton B-1956[1]	0.8 qt.	
	Ferbam, 76% wettable powder	5 lb.	4 cover sprays
	Wettable sulphur	15 lb.	
	Triton B-1956	0.8 qt.	
3	Lime sulphur	8 gal.	Pre-pink, pink and full bloom
	Colloidal Spray Modifier[2]	0.8 qt.	
	Ferbam, 76% wettable powder	5 lb.	4 cover sprays
	Wettable sulphur	15 lb.	
	Colloidal Spray Modifier	0.8 qt.	
	DDT, 50% wettable powder[3]	6 lb.	
4	Ferbam, 76% wettable powder	5 lb.	All sprays
	Wettable sulphur	15 lb.	
	DDT, 50% wettable powder[3]	6 lb.	
5	Ferbam, 76% wettable powder	5 lb.	All sprays
	Wettable sulphur	15 lb.	
	DDT, 50% wettable powder[3]	6 lb.	
	Triton B-1956	0.8 qt.	
6	Colloidal sulphur, 40% colloidal suspension	25 lb.	Pre-pink, pink and full bloom
	Triton B-1956	0.8 qt.	
	Colloidal sulphur, 40% colloidal suspension	25 lb.	4 cover sprays
	Ziram, 76% wettable powder	5 lb.	
	Triton B-1956	0.8 qt.	
7	No sprays applied (check)		

[1] Rohm and Haas Co., Philadelphia, Pa.
[2] Colloidal Products Corp., San Francisco, Calif.
[3] DDT added in first two cover sprays only.

were arranged as follows: *Top*, 8/64 in.; *centre*, 6/64 in., *bottom*, 3/64 in. For the last two spray applications the nozzle orifices were arranged thus: *Top*, 6/64in.; *centre*, 8/64 in., *bottom*, 3/64 in.

Details regarding materials applied to the various plots are given in Table 1. Although it was windy on several occasions spray applications were generally satisfactory. Dates of spray applications are shown in Table 2.

TABLE 2.—DATES OF SPRAY APPLICATIONS

Application	Sewell Orchard	Fransen Orchard
Pre-pink	May 12-13	May 13
Pink	May 19	May 19
Full Bloom	May 26	May 27
First Cover	June 9	June 8
Second Cover	June 23	June 23
Third Cover	July 5	July 5
Fourth Cover	Aug. 9	Aug. 9

Sampling and Analysing

Leaf samples were taken, after the first and second cover sprays, from all plots for sulphur deposit determinations. In addition, leaf samples were taken, after the first and second cover sprays, from Plots 3, 4 and 5 for DDT deposit determinations. Tree-top samples were taken at 15 feet and tree-bottom samples at 6 feet above ground level. Sample size, sampling technique, sample treatment and method of sulphur analysis were the same as those reported by Waddell and McArthur (6) DDT was determined by a modified Schecter-Haller procedure (2).

At harvest time, 1,000 or more McIntosh apples were picked at random from each plot and examined for scab. Apples showing any sign of infection were classed as scabbed.

RESULTS AND DISCUSSION

The presence of ferbam interfered with the method used in determining sulphur and the results from sulphur analysis were not considered reliable. There were, however, no complications with DDT and the average DDT deposits for the three plots where this material was applied are given in Table 3.

The data show that the addition of a surfactant to the spray mixture had little effect on the amount of DDT, (and, presumably, fungicide) deposited on the foliage of the trees.

Following the third lime sulphur spray which was applied during cool, humid conditions, considerable foliage injury occurred on all varieties. Northern Spy trees were most severely affected. Foliage development on McIntosh trees was arrested and the small leaves were severely damaged. In plots which were sprayed with lime sulphur plus surfactant, injury was somewhat more severe.

There was a heavy primary infection of apple scab in both orchards in Plot 6 where colloidal sulphur plus surfactant was applied. Apparently

TABLE 3.—AVERAGE DEPOSITS OF DDT ON FOLIAGE OF APPLE TREES FROM DDT-FUNGICIDE CONCENTRATE SPRAY MIXTURES. (MEANS OF 10 OBSERVATIONS)

Plot	Material	Amount per acre	First cover		Second cover	
			Top mmg./ sq. cm.	Bottom mmg./ sq. cm.	Top mmg./ sq. cm.	Bottom mmg./ sq. cm.
4	Wettable sulphur Ferbam DDT	15 lb. 5 lb. 6 lb.	3.4	4.7	2.8	5.3
3	Wettable sulphur Ferbam C.S.M. DDT	15 lb. 5 lb. 0.8 qt. 6 lb.	3.9	4.0	3.5	4.5
5	Wettable sulphur Ferbam Triton B-1956 DDT	15 lb. 5 lb. 0.8 qt. 6 lb.	3.7	3.6	2.9	3.7

the colloidal sulphur exerted little fungicidal effect at the low temperatures which prevailed during the early part of season. Ziram was added to the colloidal sulphur and surfactant in the four cover sprays to check further development of apple scab in these plots.

Following about six weeks of frequent and prolonged showers in mid-summer, non-sprayed check trees were partially defoliated and all fruits on these trees were infected with apple scab. In plots where ferbam plus wettable sulphur had been applied, the fruit carried a blotchy deposit. Where a surfactant had been added, however, the deposit was much more uniform and less obvious. In Plot 6, where sprays of ziram plus colloidal sulphur plus surfactant had been applied, the deposit was practically invisible.

At harvest time, McIntosh fruits in both orchards showed about 2 per cent spray injury where ferbam plus wettable sulphur plus surfactant had been applied. The injury was limited to apples within about 6 feet of the path of the sprayer nozzles. Where the surfactant Triton B-1956 had been added to the spray mixture of ferbam plus wettable sulphur, the injury occurred under or around the margin of a heavy circular deposit of fungicide on the underside of the fruit farthest from the sprayer. Where the surfactant Colloidal Spray Modifier, hereafter referred to as "C.S.M.", had been added, the injury was at the margin of an irregularly-shaped blotch of fungicide extending around the calyx end of the fruit. No injury occurred in the plots sprayed with colloidal sulphur plus ziram plus surfactant.

Since the addition of surfactants to the fungicidal sprays had little effect on the amount of spray material deposited (Table 3), apparently the reduction in scab infection was largely due to the more uniform deposit obtained when surfactants were added to the spray mixtures.

To judge from the experiment, it seems probable that the addition of a suitable non-ionic surfactant will measurably improve the effectiveness of the spray mixtures most commonly used for controlling apple scab in British Columbia. Before there can be a general recommendation for a surfactant in such spray mixtures, however, further experimental work is required in order to determine how to lessen the likelihood of spray injury

TABLE 4.—EFFECT ON APPLE SCAB INFECTION OF NON-IONIC SURFACTANTS IN CONCENTRATE FUNGICIDAL SPRAY MIXTURES[1]

Plot	Materials applied	Stage of application	Scabby fruit %		
			Sewell	Fransen	Average
1	Lime sulphur	Pre-pink, pink and full bloom			
	Ferbam Wettable sulphur	4 cover sprays	10.9	12.5	11.7
2	Lime sulphur Triton B-1956	Pre-pink, pink and full bloom			
	Ferbam Wettable sulphur Triton B-1956	4 cover sprays	5.2	3.0	4.1
3	Lime sulphur C.S.M.	Pre-pink, pink and full bloom			
	Ferbam Wettable sulphur C.S.M.	4 cover sprays	5.6	3.1	4.3
4	Ferbam Wettable sulphur	All sprays	10.4	16.7	13.5
5	Ferbam Wettable sulphur Triton B-1956	All sprays	6.8	8.6	7.7
6	Colloidal sulphur Triton B-1956	Pre-pink, pink and full bloom			
	Colloidal sulphur Ziram Triton B-1956	4 cover sprays	7.5	17.6	12.5
7	No spray applied (check)		100.0	100.0	100.0

[1]Results based on count of 1,000 apples from McIntosh trees in each plot at harvest time.

There is reason to believe this can be accomplished by reducing the amount of water in the spray concentrates. Fifty gallons of spray liquid per acre may suffice instead of about 75 gallons or more as is now common practice.

ACKNOWLEDGEMENTS

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SUSPECTED ICE-GIRDLING FOLLOWING THE MOUNDING OF YOUNG PEACH TREES IN SOUTHWESTERN ONTARIO[1]

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ABSTRACT

Some doubt exists regarding the occurrence of ice-girdling in young peach trees mounded with soil prior to winter. Several instances of injury have been noted recently which were very suggestive of ice pressure, judging by the type and the location, above ground level, of the bark and cambium damage. Surveys made in two mounded orchards in 1948 and 1953 disclosed that more than 28 per cent of the trees suffered moderate to severe injury of this type with some trees failing to survive. As a result the practice of mounding young peach trees is being discouraged without any apparent harmful effect.

INTRODUCTION

Young and old peach trees in southwestern Ontario are commonly mounded with soil to heights varying from 6 to 12 inches above ground level from mid-September to the latter part of November. The early mounding is associated with the use of paradichlorobenzine for borer control but ethylene dichloride applied in mid-October is now being used more extensively. With the increasing use of DDT borer sprays applied in mid-summer to young trees and the necessity for the ethylene dichloride treatment only once in 3 years on older trees, it becomes necessary to assess mounding purely from a cultural standpoint since the practice is still followed in the absence of any borer treatment. Several instances of severe lower trunk injury have been observed recently on young mounded peach trees. Two orchards were examined to determine the extent and the possible nature of the injury.

REVIEW OF LITERATURE

Chandler (2) and Gardner, Bradford and Hooker (3) allude to the frequent occurrence of collar injury at ground level in peaches which they variously attribute to immaturity of tissues known to mature last in the area of the trunk. No mention of the possibility of mechanical damage by ice is made in either treatise but tissue immaturity could be a factor since such tissues might be more readily damaged by pressure of any sort. In addition, early mounding, i.e. in mid-September, might delay maturation by insulating the enclosed bark area from the hardening influence of cooler night temperatures.

Bradford and Cardinell (1) in a survey of winter injury in Michigan refer to reports of ice-girdling in 1885 and 1911 in that State. Much controversy centred about the advisability of mounding young peach trees because of the necessity for borer control, but it was generally agreed that water should not be allowed to collect at the base of the tree. Johnston (4) summarized these observations and implied that mounding older peach trees in Michigan served no useful purpose and that young peach trees

[1] Contribution No. 850 from the Horticulture Division, Experimental Farms Service.
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could be severely injured by the action of ice if mounded in the fall. No experimental evidence was found in the literature which would indicate that the mounding of peach trees was necessary, other than as required for borer control and the discouragement of mice.

MATERIALS AND METHODS

Two young orchards belonging to Mr. Tillotson, Harrow, and Mr. Driedger, Leamington, provided the trees. The Tillotson orchard was planted in 1946 on a ridge of sandy loam soil and mounded prior to the onset of winter without the use of chemicals for borer and rabbit control. A number of trees were injured during the winter and several died during 1947 but not until May 1948 was a survey of the orchard made to determine the extent of injury. Similar injury was reported in the summer of 1952 in the Driedger orchard which had been planted in the previous year (1951) and mounded in the fall without the incorporation of any chemicals. A survey of this orchard was made in March, 1953. Trees in both orchards were rated as to the degree of injury and placed in the following major categories: *Healthy*, *Moderate Injury*, *Severe Injury*, and *Dead* (Figure 1). In the Driedger orchard a commercial asphalt compound was applied in 1952 to all injured trees, including those suffering only a roughening of the bark. The presence of emulsion in 1953 on an otherwise healthy tree was an indication that such damage was only superficial and it was recorded.

RESULTS

Tillotson Orchard, 1946-1947.

The tree examination made in May 1948 showed that the injury must have occurred some time during the dormant season of 1946-1947. The characteristic injury was confined to a point on the trunk which corresponded with the top of the mound of soil placed there in the fall of 1946 (Figure 1). On severely injured and dead trees the bark appeared to have been loosened and pushed upwards in the fashion of a concertina by some form of pressure (Figure 1,*C*). Other trees suffered moderate injury (Figure 1, *B*) and some were healthy in appearance except for a slight roughening of the bark, (Figure 1, *A*). The results of the examination are presented in Table 1.

TABLE 1.—SUSPECTED ICE INJURY TO YOUNG MOUNDED PEACH TREES WHICH OCCURRED DURING THE WINTER OF 1946-1947. (TILLOTSON ORCHARD)[1]

Variety	No. of trees	Healthy	Moderate injury	Severe injury	Dead,[2] Missing
		%	%	%	%
Golden Jubilee	78	57	23	3	17
Redhaven	80	60	23	6	11
Halehaven	74	51	30	7	12
McGuigan	74	80	11	1	8
Elberta	73	60	27	7	6
Average		62	23	5	11

[1] Orchard planted 1946.
[2] Cause of death or absence not definitely known.

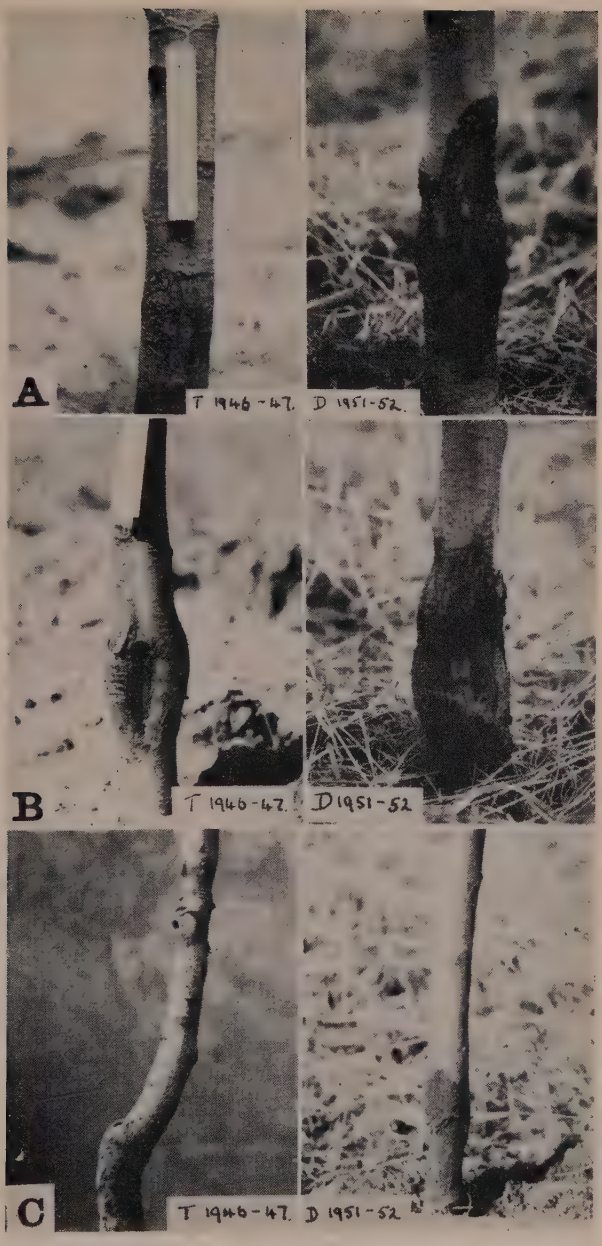

FIGURE 1. Suspected ice-girdling of peach trees in southwestern Ontario. *Left*, Tillotson Orchard. *Right*, Driedger Orchard. *A*—Healthy, roughened bark. *B*—Moderate injury. *C*—Dead. (*Photos taken May 1948 and March 1953*)

Sixty-two per cent of the trees were rated as healthy, with 23 per cent suffering moderate injury and expected to survive. Only 5 per cent of the trees were rated as severely injured, but it is believed that many of the missing and dead trees would have been placed in this category if the examination had been made in 1947. If this assumption is correct, then mounding these young trees caused injury to more than 28 per cent of the stand.

The McGuigan variety suffered considerably less injury than the other varieties. Seventeen per cent of the Golden Jubilee variety were listed as dead and missing which may have been partially due to the location of some trees in a low area.

The exact date of mounding in the fall of 1946 was not available for this orchard but the work was believed done in October. The lowest temperature recorded during the 1946-1947 winter was 1·5° F. in February.

Driedger Orchard 1951-1952

A similar type of injury caused the loss of some trees in the Driedger Orchard in 1951-1952. Table 2 shows the results of a survey made on a total of 320 trees in this orchard in March, 1953.

TABLE 2.—SUSPECTED ICE INJURY TO YOUNG MOUNDED PEACH TREES WHICH OCCURRED DURING THE WINTER OF 1951-1952. (DRIEDGER ORCHARD)[1]

Variety	No. of trees	Healthy		Injured		Dead,[2] Missing
		Bark smooth	Bark rough	Moderately	Severely	
		%	%	%	%	%
Golden Jubilee	120	13	47	18	9	15
Redhaven	100	29	45	16	8	2
Halehaven	100	5	58	27	8	2
Average		16	50	20	8	6

[1] Orchard planted 1951.
[2] Cause of death or absence not definitely known.

The extent of moderate and severe injury (Figure 1, *B* and *C*) was comparable with that noted in the Tillotson orchard and involved 28 per cent of the stand. Only 16 per cent of the trees were free of some manifestation of injury, with 50 per cent of the entire planting suffering superficial injury resulting in a roughening of the bark. As in the previous orchard no chemicals had been placed beneath the mound for borer control and the trees had not been treated with a rabbit repellent. The trees were mounded some time during October, 1951 and the winter's low temperature of −3° F. occurred in December.

DISCUSSION

After examining more than 100 injured trees from two affected orchards the following explanations for the injury are advanced: The young peach tree, whipping around in a mound of comparatively loose soil, may form

a cone which subsequently freezes and may fill with moisture. It is suggested—in the absence of any definite proof—that the ice which forms first in the mound becomes firmly attached to the bark. As ice formation continues in the cone the resulting expansion may lift ice already formed and thus cause the damage. The injury associated with the presence of ice might also be due to the withdrawal of water by plasmolysis from the cell tissues of the outer bark and cambium as described by Gardner, Bradford and Hooker (1) in their discussion on the effects of freezing. The wrinkling of the bark above the damaged area suggests, however, an upward or lifting action and can be seen clearly in Figure 1,*C* (*left*).

The exact dates of mounding were not available for either orchard but since chemicals were not used, mounding was delayed until October by which time the trunk and lower limbs should have matured thus reducing the possibility of tissue immaturity as the main cause of damage. In addition, the injury illustrated in Figure 1 was rarely more than 2 to 3 inches in extent and invariably corresponded with the top of the mound where tissue maturation would be least affected by the insulating effect of the soil.

The McGuigan variety in the Tillotson orchard suffered 20 per cent injury of all kinds as compared with 49 per cent and 95 per cent injury for Halehaven in the Tillotson and Driedger orchards respectively. Redhaven trees were intermediate in susceptibility in both orchards, with Golden Jubilee trees suffering a higher percentage of dead and missing trees than any other variety under observation. These differences in varietal susceptibility to ice-injury may be related to the firmness of the outer layer of the bark.

Weather records for the two winters in question, 1946-1947 and 1951-1952, showed that they were both preceded by high temperatures in October but were not characterized by severe drops in temperature during the colder months. Acting on suggestions from the Harrow Experimental Farm, a number of young orchards have not been mounded in recent years. Careful examination of such orchards has not disclosed any injury which might be attributable to the absence of a mound of soil.

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THE DEVELOPMENT OF THE DIGESTIVE ENZYME SYSTEM OF THE PIG DURING ITS PRE-WEANING PHASE OF GROWTH[1]

A. PANCREATIC AMYLASE AND LIPASE

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ABSTRACT

The amylolytic activity of crude aqueous extracts of the pancreatic glands of suckling pigs increases markedly with advancing age. The lipolytic activity of such extracts is of a high order at birth and remains high as growth proceeds. Consideration of these findings is a prerequisite to the formulation of diets containing starch and fats.

INTRODUCTION

It has been demonstrated (9) that the suckling pig can rarely express its full growth potential if it must depend solely on the milk output of the sow for its nutrient requirements. It is essential that the sow's milk be supplemented with a suitable creep ration. In the formulation of such rations little or no attention has been given to the amenability of the ration constituents to degradation by the digestive enzyme complex of the young pig. The transition from a milk diet to one containing a great variety of complex nutrient substances implies that there is a shift in the relative importance of the various digestive enzymes specific to the particular substrates involved. It is reasonable that such a shift be accompanied by changes in the nature and quantity of the various digestive secretions. In an attempt to establish the presence or absence of such changes in the enzymatic spectrum and to delineate their nature, a study has been made of the amylase and lipase content of the pancreatic glands of young pigs at various ages from birth to weaning at seven weeks.

MATERIALS AND METHODS

(a) Removal of Gland

Pairs of purebred Yorkshire pigs ranging from birth to 7 weeks of age were randomly selected from two litters. They were sacrificed by severance of the jugular veins. The pancreatic glands were immediately removed and freed of all visible fat and extraneous tissue. A representative portion was taken from each for moisture determination and the remainder frozen. The glands remained in the frozen state (−10° C.) until ready for assay.

[1] Contribution from the Division of Animal Science.

[2] Graduate Student; recipient of a National Research Council of Canada Bursary while engaged in this investigation.

TABLE 1.—THE AMYLOLYTIC ACTIVITY OF PANCREATIC GLAND EXTRACTS FROM SUCKLING PIGS RANGING IN AGE FROM BIRTH TO 37 DAYS.

Pig No.	Age in days	Body weight		Pancreas		Amylase units per gram of gland		Units per kg. body weight
		lb.	kg.	Weight gm.	Per cent dry matter	Wet	Dry	
1 and 2*	1	2·8	1·27	1·28	20·00	91	456	92
3	6	4·9	2·23	3·60	22·20	893	4030	1448
4	11	6·7	3·05	4·47	22·40	926	4290	1356
5	22	14·4	6·55	7·05	26·80	1561	5870	1682
6 and 7	37	22·6	10·25	16·40	27·20	2602	9645	4296

* Glands from two pigs pooled for enzyme extract preparation.

TABLE 2.—THE LIPOLYTIC ACTIVITY OF PANCREATIC GLAND EXTRACTS FROM SUCKLING PIGS RANGING IN AGE FROM BIRTH TO 49 DAYS.

Pig No.	Age in days	Body weight		Pancreas		Lipase units per gram of gland		Units per kg. body weight
		lb.	kg.	Weight gm.	Per cent dry matter	Wet	Dry	
1 and 12	0	2·9	1·3	1·0	22·7	76	335	51
5	7	4·0	1·8	2·8	25·0	62	248	92
2 and 9	14	6·2	2·8	3·8	26·7	84	315	111
4 and 14	21	13·0	5·9	7·0	29·1	70	240	82
15	35	20·0	9·0	10·1	25·4	91	358	101
7	49	34·0	15·3	17·1	27·4	168	615	186

(*b*) *Method of Preparation of Enzyme Extracts*

(i) Amylase extract

A crude extract was obtained by grinding the gland in a mortar with twice its weight of alumina powder*. The ground gland was then extracted with a 0.067 M phosphate buffer (pH 7). The extract thus obtained was diluted with buffer to a volume such that each ml. of enzyme extract represented 20 mgm. of original wet tissue. This preparation was used for the determination of amylase activity.

(ii) Lipase extract

The pancreatic tissue was ground to a fine paste with washed quartz sand. The slurry so obtained was filtered through eight layers of cheesecloth. The filtrate was diluted with water so that each ml. of extract represented 17 mgm. of glandular tissue. This solution was used for the determination of lipolytic activity.

(*c*) *Method of Assay*

(i) Amylase activity

The enzymatic activity of the extract was assayed using a modification of the Wohlgemuth procedure proposed by Koch and Hanke (7). This method was further modified to the extent that 30 mgm. sodium chloride per 100 ml. of reaction mixture were added. This salt has been reported to permit maximum activation of the enzyme (6). Enzymatic activity is expressed as the number of ml. of 2 per cent starch solution hydrolysed by 1 gm. of pancreatic tissue in 30 minutes at 40° C.

(ii) Lipase activity

The activity of this extract was assayed using the method developed by Goldstein *et al.* (5). This procedure was modified to the extent that fat** from sows' milk replaced tributyrin as the substrate. An emulsion stable for several hours was obtained by passing the substrate mixture through a hand homogenizer.

EXPERIMENTAL RESULTS

The number of units of amylase activity per unit weight of gland expressed on both a dry and a wet basis increases with increasing age and body weight. The number of activity units per kilogram body weight also increases markedly with age. This is particularly evident after the age of 22 days. The results of the amylolytic activity determinations are summarized in Table 1 and Figure 1.

The results of the determination of the lipolytic activity of the pancreatic gland extracts expressed as lipase units per gram of wet tissue and as lipase units per kilogram body weight are summarized in Table 2 together with the pertinent growth data.

* Alumina powder A-303, obtained from the Aluminum Ore Co., East St. Louis, Ill.

** The sow milk fat was provided by C. P. McMeekan, Ruakura, Hamilton, New Zealand.

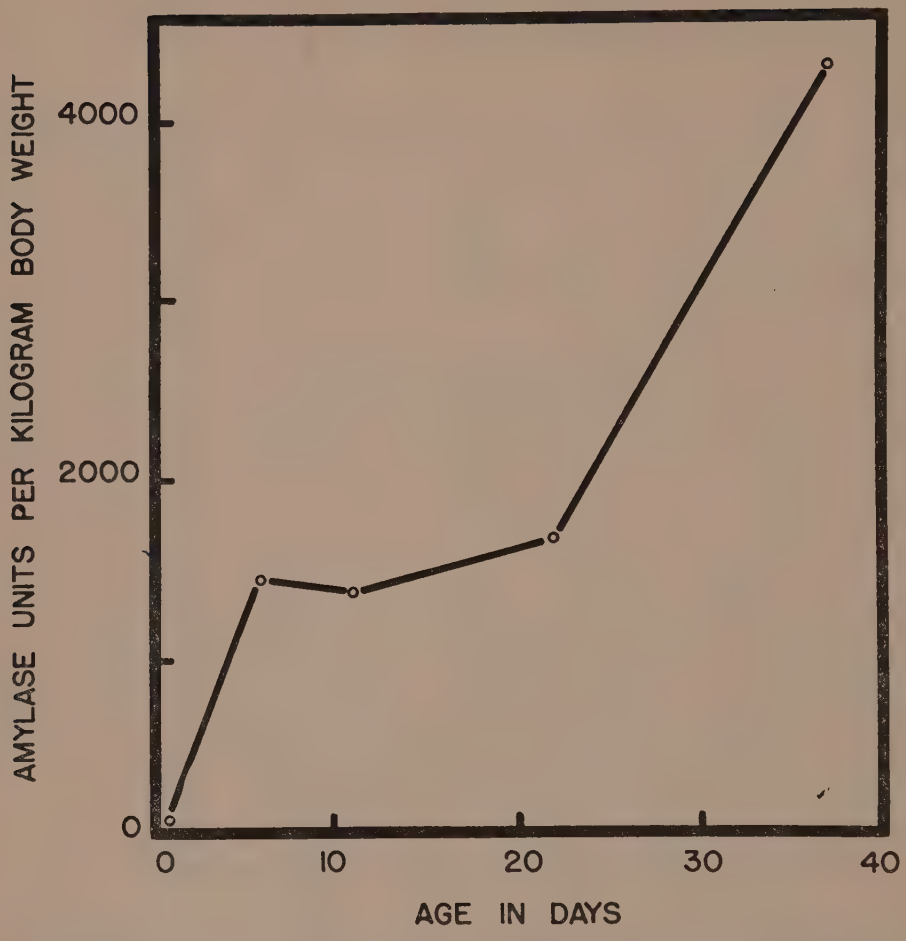

FIGURE 1. The amylolytic activity of pancreatic glands per unit body weight of suckling pigs ranging in age from birth to 37 days.

DISCUSSION AND CONCLUSIONS

Although the number of pigs used is small and may not constitute a representative sample of the swine population, the results obtained do tend to support the concept that a transition occurs in the nature of the digestive secretions of growing pigs. The amylolytic activity of the pancreas per unit weight of gland is negligible in the newborn pig. Subsequent to this time the activity values increase rather markedly to 37 days of age (Table 1).

Since the size of the pancreas relative to body weight could decrease with age and thus in part compensate for the increase in amylase activity of the gland, a more useful method of expressing the data is in terms of units of enzyme activity per kilogram body weight. When they are represented in this way (Table 1, Figure 1), the results obtained are qualitatively similar to those presented above. It is apparent, therefore, that

if the amylolytic activity of a crude gland extract truly reflects the ability of the pancreas to produce alpha-amylase, then the starch hydrolysing capacity of the pig increases with increasing age over the period studied.

There is a suggestion from the data represented in Figure 1 that amylase production undergoes a significant increase at or about the fourth week of life. It is tempting to speculate that this increase arises in response to the consumption of feed constituents other than those found in milk. Although this supposition cannot be substantiated from the present data, it implies that the digestive secretions may alter in a quantitative sense in response to changes in the nature of the diet. Regardless of the predisposing causes for these changes, it is apparent that in the formulation of rations for the young pig, due recognition should be made of the changing enzyme pattern.

In the present work no attention has been given to the output of salivary amylase. It is generally agreed (1) that the production of amylase by the pancreas far exceeds that of the salivary glands.

Lipolytic activity of the digestive system of the young pig is of a high order at birth and remains high with advancing age. Assuming that the values for lipase activity found in this study are a valid index of lipase production, the potential lipid hydrolysing capacity of the intact animal is also high.

If partial or complete hydrolysis of dietary lipids must precede absorption, the above findings are significant in relation to the feeding of young pigs. From the recent literature, however, there is some indication that the importance of the lipolytic digestive enzymes is not as great as once suspected. Under normal *in vivo* conditions it has been established that little ingested fat is completely hydrolysed to glycerol and free fatty acid prior to absorption (4, 8). Furthermore, it would appear that the breakdown of triglycerides need not precede absorption for, in the absence of lipolysis, absorption may still occur (3). Absorption of lipid materials can take place through the lacteals of the lymphatic system. In order that such absorption may occur, the lipids must be emulsified to give a particle diameter of less than 0·5 microns (2). The only system which provides spontaneous emulsification over a sufficiently wide pH range is the system monoglyceride/bile salt/fatty acid (2). It is apparent that two components of this system—monoglyceride and fatty acid—are hydrolytic products of the tri-glycerides. In order, therefore, to permit efficient, rapid emulsification of fats, preliminary hydrolysis should occur, at least to a limited extent. Since the simpler products of hydrolysis (fatty acids and glycerol) may be absorbed into the portal system and the more complex fractions (mono-, di-, and tri-glycerides) are absorbed by way of the lymphatic system, the extent of hydrolysis determines the route of absorption (3). Therefore, although lipid hydrolysis need not precede absorption, a certain degree of lipolysis will facilitate emulsification and provide more rapid and complete absorption. To this extent, lipolytic enzymes are important prerequisites to fat utilization. The results of this investigation indicate that over the age range studied, lipase hydrolysing enzymes are relatively abundant in the gut of the pig.

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THE DEVELOPMENT OF THE DIGESTIVE ENZYME SYSTEM OF THE PIG DURING ITS PRE-WEANING PHASE OF GROWTH[1]

B. INTESTINAL LACTASE, SUCRASE AND MALTASE

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ABSTRACT

A quantitative and qualitative expression of the changes that occur in the saccharolytic enzyme complex of the suckling pig has been attempted. Intestinal lactase, sucrase, and maltase activity were measured using the excised small intestines of suckling pigs taken at various ages from birth to weaning. Lactase activity was shown to be of a high order from birth through the first 2 weeks of life. At or about this time a precipitous decline in activity was noted which reached minimal levels after 3-4 weeks. Sucrase and maltase activity were found to increase from negligible levels at birth to a maximum after about 25 days. These observations clearly demonstrate that important changes do occur in the digestive enzyme complex as early growth proceeds. Such enzyme changes must markedly affect the suitability of different carbohydrates as sources of energy for the growing animal.

INTRODUCTION

Few systematic studies have been made of the changes which may occur in the digestive enzyme complex of animals as growth and development proceed. Plimmer (18) has reviewed the limited early work on the occurrence of intestinal lactase. More recently Koehler and Allen (17) have pointed out that, although the presence of lactase in infancy is indicated, evidence as to the occurrence of lactase activity in the intestines of the human adult is meagre. Cajori (4, 5) has shown that the lactase activity of the intestine of the dog is intimately associated with the mucosal cells and that the jejunal mucosa exhibited a 10 to 30 per cent greater activity than the duodenal mucosa. Heilskov (14) found that the lactase activity of bovine and rabbit small intestine is much higher in young than in older animals. Kitts *et al.* (16) have shown that the pancreatic amylase activity of pigs increases from negligible levels at birth to high levels after 21 days. Plimmer and Rosedale (19) and Hamilton and Mitchell (12) failed to demonstrate lactase activity in chicken intestine.

Circumstantial evidence is available to indicate, in the case of the pig, that marked changes occur in the digestive enzymes during the first few weeks of life. It has been shown that the day-old pig is unable to survive on milk replacement formulae in which sucrose and d-fructose served as the source of dietary carbohydrate (3). Survival was satisfactory when d-glucose or invert sugar replaced the sucrose or fructose. Other experiments have demonstrated that the survival of week-old pigs on sucrose diets is somewhat more satisfactory (2). When weanling pigs are offered diets in which the carbohydrate source is lactose, growth is impaired and a moderate diarrhoea develops (1). At this age, sucrose as the carbohydrate source permits satisfactory growth. Fischer and Sutton (10)

[1] Contribution from the Division of Animal Science.

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and others (7, 11, 23) noted similar effects when rats are offered a high lactose diet. The apparently anomalous behaviour of lactose when fed to older animals has been the subject of an excellent review by these authors (8).

On the basis of the papers reviewed above it seems reasonable to postulate that the digestive enzymes undergo a gradual quantitative shift with age from birth. The work reported in the present paper was carried out to test the validity of this postulate and, if possible, to give it quantitative expression.

MATERIALS AND METHODS

(a) *Animals*

Randomly selected pairs from two litters of purebred Yorkshire pigs were sacrificed at birth and at various ages to weaning for the preparation of intestinal homogenates. The pigs were not offered a creep ration although they had access to their sow's feed at all times. The growth data for the two litters are given in Table 1 with information on the changes in the mass, length and moisture content of the small intestines.

(b) *Removal of Organs*

The animals were sacrificed by severance of the jugular veins. The small intestines were removed immediately and freed of all visible fat and other extraneous tissue. The contents were flushed out with cold running water. A portion was removed for moisture determination and the remainder was frozen. The tissue remained frozen (−10° C.) until ready for assay.

TABLE 1.—GROWTH AND TISSUE DATA ON THE EXPERIMENTAL ANIMALS

Pig No.	Age in days	Sex	Body weight kg.	Intestine		Intestine length cm.
				Weight gm.	Per cent dry matter	
			Litter 1			
1	0	M	1·0	39·1	19·3	320
2	0	F	1·1	52·9	15·2	366
4	7	M	2·5	103·2	18·8	549
9	7	F	3·1	130·1	18·3	517
3	14	F	5·3	188·8	19·5	732
10	14	M	5·0	157·5	18·9	732
5	21	F	6·8	187·5	18·5	820
8	21	M	7·0	154·6	18·0	765
7	36	M	13·5	262·9	19·5	991
6	36	F	11·8	242·2	19·4	935
11	51	M	20·0	366·9	16·8	1220
			Litter 2			
12	0	F	1·2	20·3	18·3	316
1	0	M	1·4	31·2	18·1	352
5	7	M	1·8	52·6	17·0	415
13	7	M	1·6	46·0	18·4	430
9	14	F	2·7	86·7	18·0	552
2	14	M	2·9	80·0	18·4	521
14	21	M	5·1	161·0	17·7	729
4	21	M	6·7	187·2	18·4	754
15	35	M	9·0	271·1	19·0	888
7	49	M	15·3	347·2	27·5	936

(c) *Method of Preparation of Enzyme Extracts*

After division of the intestine into three equal sections extracts were prepared from each. In this way an evaluation of changes in enzyme activity along the intestine could be obtained while still permitting an expression of the total activity for each animal. After comminution of each section, a representative 15-20 gram aliquot was homogenized by grinding with washed quartz sand. The homogenate was transferred into a 250 ml. flask using distilled water. Ten ml. of toluene were added and the mixture allowed to incubate for 24 hours at room temperature with frequent shaking. The autolysate so obtained was strained through 8 layers of cheesecloth, then diluted with distilled water to a volume such that each ml. of enzyme preparation represented 100 mgm. of intestine. This preparation was used for the determination of lactase, sucrase and maltase activity.

(d) *Methods of Enzyme Assay*

The hydrolytic capacity of the enzyme preparations for lactose, sucrose and maltose was assessed using the method proposed by Heilskov (13). Since this worker did not determine sucrase or maltase activity, it was necessary to modify his method to the extent that the pH of the reaction mixture was adjusted to 7·0 for maltase (22) and to 4·5 for sucrase* (21). In the case of all three enzymes, the activity is expressed in terms of weight of the respective disaccharide hydrolysed in 2 hours at 37° C. This represents a departure from Heilskov (13) in the method of expressing enzyme activity.

EXPERIMENTAL RESULTS

The results of the determinations of the hydrolytic activity of the small intestine with respect to the three substrates are summarized in Tables 2 and 3 and expressed graphically in Figure 1.

The values reported in Tables 2 and 3 represent averages where more than one animal was sacrificed at any given age. The determination of maltase activity was conducted on the intestines from the second litter of pigs only.

* Since this work was completed, other experiments on swine and beaver sucrase have shown that the pH optimum for intestinal sucrase appears to be in the neighbourhood of 7 rather than 4·5. This finding does not alter the qualitative picture presented here. The levels of sucrase in the units used should be approximately three times greater than those shown.

TABLE 2.—AVERAGE TOTAL POTENTIAL WEIGHT OF SUGAR HYDROLYSED IN TWO HOURS PER KILOGRAM BODY WEIGHT.

Age in weeks	Gm. lactose		Gm. sucrose		Gm. maltose
	Litter 1	Litter 2	Litter 1	Litter 2	Litter 2
Newborn	10·4	4·8	0·0	0·0	0·0
1	13·5	6·2	0·9	1·3	2·0
2	12·6	7·3	2·1	1·5	3·1
3	4·9	10·1	2·2	2·1	4·3
5	2·5	3·5	2·0	2·9	5·3
7	1·0	2·2	1·6	3·3	5·0

DISCUSSION AND CONCLUSIONS

It is apparent from the results of the present investigation that the activity of the intestines of young pigs with respect to the hydrolysis of lactose, sucrose and maltose undergoes significant changes with age prior to weaning. Reference to Figure 1 and Table 2 shows that lactase activity is high and sucrase and maltase activity low during the first few days of life. Lactase activity reaches a peak at roughly three weeks of age. Subsequent to this time there is a precipitous decline to minimal levels at four to five weeks. Sucrase and maltase activity, on the other hand, rise steadily from negligible levels at birth to significant levels in one to two weeks. If the lactase, sucrase and maltase activity of aqueous extracts of swine small intestine reflect true differences in the sugar hydrolysing potential of intact animals, then the above results are of fundamental importance to nutritional studies.

Variations in the physiological effects of different dietary carbohydrates have been reported on numerous occasions. Some of the criteria of response have been growth, feed efficiency, livability, and the production or recession of undesirable effects. The consensus of the results obtained indicate that very young animals are unable to utilize sucrose as a dietary carbohydrate but may utilize lactose. The reverse situation holds true for older animals. A plausible explanation for these facts is afforded by the results of the present investigation. That is, the absence of sucrase in the intestine of young pigs could account for the failure of investigators to formulate successful milk replacement formulae based on sucrose. Further, the sharp decline in lactase activity in older animals could explain the deleterious effects of lactose for such animals. Support for this contention is provided by the work of Riggs and Beaty (20) and of Ershoff (6) who have demonstrated that equimolar mixtures of glucose and galactose do not produce the diarrhoea which characterizes lactose feeding in older animals. Fischer and Sutton (8) proposed that lactose diarrhoea is not induced by a direct reaction of the intestine to this sugar. Rather, it exerts a "hydragogue" effect. That is, the unabsorbed lactose causes an increased osmotic concentration in the lumen of the gut. Active secretion of water into the gut in response to this increased osmotic pressure induces a watery diarrhoea. Their concept receives further support from the results presented here for it has been noted that the ages at which diarrhoea appears in response to the feeding of lactose and sucrose correspond to the ages at which the respective enzymes were found to be absent or nearly absent in the experimental animals. Final proof on this point, however, awaits further experimentation. Finally, it has been common experience that young pigs are reluctant to consume creep rations until 14 to 21 days *post partum*. The present results and those of the previous work in this series (16) suggest that this reluctance may be based on the inability of such animals to hydrolyse starch, sucrose or maltose until the necessary enzymes are formed in appreciable amounts.

Reference to Table 3 shows the activity of the three enzymes expressed in terms of the potential weight of sugar which can be hydrolysed per kilogram of body weight. This mode of expression was chosen because daily nutrient requirements are roughly proportional to body weight.

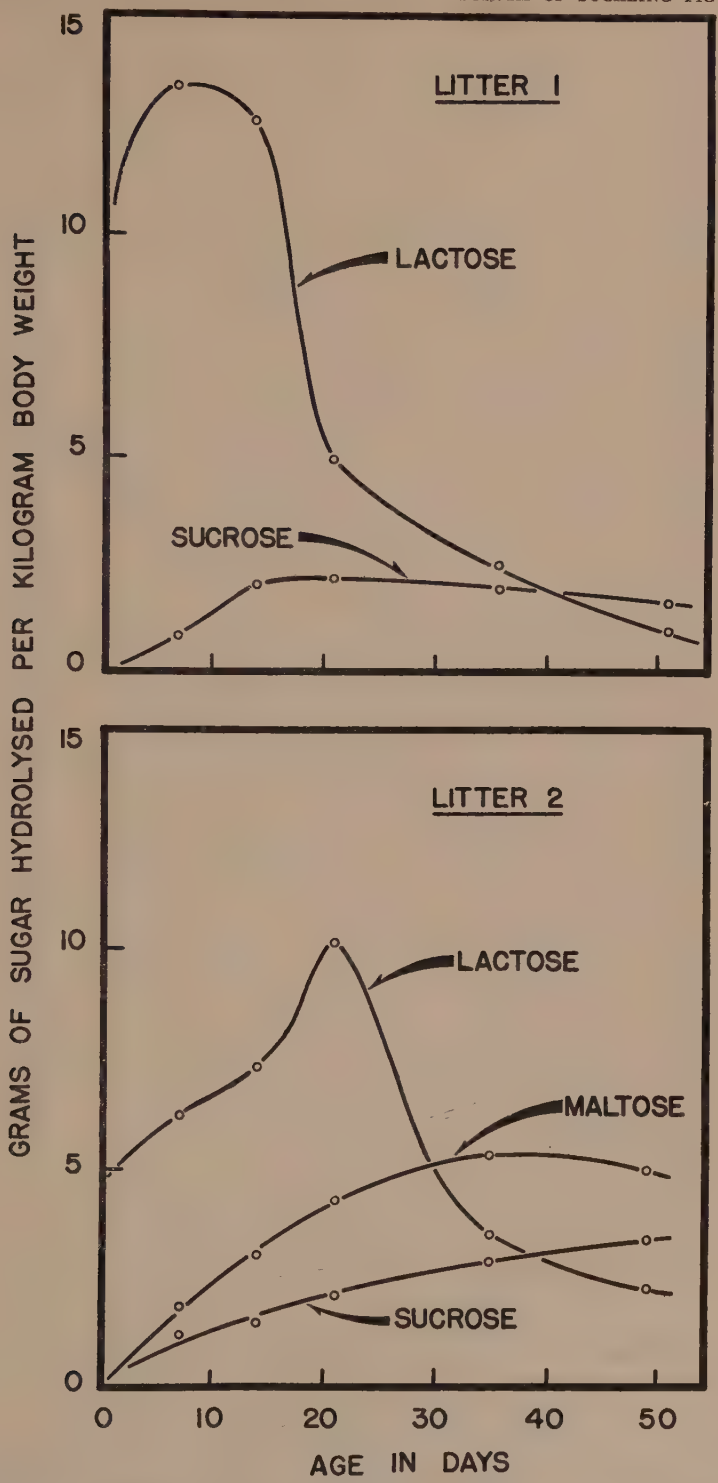

FIGURE 1. Changes in the average potential weight of sugar hydrolysed in 2 hours per kilogram of body weight.

TABLE 3.—AVERAGE WEIGHT OF SUGAR HYDROLYSED PER GRAM OF WET INTESTINE

Sugar	Age in weeks	Upper section		Middle section		Lower section	
		Litter 1	Litter 2	Litter 1	Litter 2	Litter 1	Litter 2
Lactose	Newborn	283	397	250	323	223	168
	1	279	273	439	326	225	116
	2	372	324	467	293	283	203
	3	224	330	233	332	122	177
	5	167	200	169	179	41	82
	7	83	174	52	126	22	47
Sucrose	Newborn	0	0	0	0	0	0
	1	22	74	30	51	12	26
	2	73	55	83	82	26	27
	3	85	92	108	113	72	63
	5	79	74	103	118	119	195
	7	70	153	69	155	49	226
Maltose	Newborn	—	0	—	0	—	0
	1	—	82	—	114	—	26
	2	—	140	—	156	—	58
	3	—	185	—	213	—	147
	5	—	177	—	223	—	295
	7	—	254	—	220	—	335

Hence, enzyme activity expressed as a function of body weight should provide a more valid comparison between animals. In order to assess the variations which occur along the length of the intestine, enzymatic activity has also been expressed in terms of the weight of sugar hydrolysed per unit of wet intestine for the three sections of this organ (Table 3). The age changes in enzyme activity per unit of wet intestine follow the same trend for each section. The middle third appears to possess the greatest activity. Cajori's (5) results on the intestinal lactase production of the mature dog are in essential agreement with this finding. On the other hand, Heilskov (14) has shown that the upper portion of the intestine of the growing bovine and rabbit has the highest activity. The present work may be criticized for its arbitrary division of the intestine into three equal portions in that it fails to recognize the changes in structure which occur along the walls of this organ. Further work should be carried out to afford a more precise correlation between cellular structure and enzyme activity.

As the data in Tables 2 and 3 show, there is some variation from animal to animal in the age at which activity changes occur. This result should not be unexpected in that chronological age is probably a poor baseline against which to plot enzyme changes. It would be reasonable to expect that physiological age at birth may be the controlling factor in the enzyme pattern of any given animal. In opposition to this hypothesis, however, is the possibility that the rate of development of the various enzymes might be influenced by the dietary intake of their specific substrates. That is, the pattern of enzyme secretion may, in some sense, be an adaptive process. Fischer and Sutton (9) have demonstrated that the absorption (apparent hydrolysis) of lactose takes place to a greater extent in rats that had been receiving lactose in their diet than in those on a lactose-free regimen. Other workers (10, 20, 23) have noted that older animals show degrees of adaptation when lactose is fed for an extended period. On the other hand Heilskov (14), was unable to maintain lactase production in the mucous membrane of the rabbit small intestine when dietary lactose was provided for 15 weeks *post partum*. Furthermore, in the present study it is to be noted that some enzymatic activity had appeared before the animals had access to the substrates specific to the three enzymes studied. Changes in the enzymatic spectrum, therefore, may be adaptations to a changing dietary environment or physiological alterations associated with age. It is equally possible that a combination of both factors regulate enzymatic output. If so, the extent to which each is operative could vary with age.

The results of the present investigation suggest the need for an extended quantitative study of age changes in the digestive enzyme systems of animals both on "standard" diets and under the influence of relatively large intakes of particular feed substrates. Such a study should embrace as many diverse animal species as possible and should map the age changes in digestive enzyme output over the complete growth phase. Besides being of fundamental importance, information so gained would be invaluable in the practice of animal (including human) feeding. The indirect influence of this changing enzymatic pattern on the commensal microflora of the intestine and on the response of this microflora to supplemental antibiotic feeding also merits critical attention.

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A STUDY OF FERTILITY IN DIPLOID DOLLARD RED CLOVER[1]

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ABSTRACT

Two diploid plants of red clover were selected from two apparently distinct lines of the Dollard variety (one plant from each line) to represent high and low seed yielding abilities. Crosses were made within and between the open pollinated progenies of these two plants. Factors affecting fertility were studied in the progenies produced through these crosses.

Variations in the amount of aborted pollen between and within plants in progenies of 12 crosses indicated that this type of partial male sterility is of genetic origin. The few meiotic abnormalities in the pollen mother cells accounted for only a small fraction of the aborted pollen in many of the plants and did not seem to affect seed set. One plant was found in which half of the ovules contained no mature embryo sacs at the time of anthesis.

The general mean number of seeds per head in progenies from outcrosses was significantly higher than the mean in progenies from sibcrosses. For the outcrosses, in general, the higher the parental mean value the higher the progeny mean, and seed yields of progenies from High × High crosses were significantly better than from High × Low ones. For the sibcrosses, in general, the mean progeny yield was not predictable from the parental mean value but here also seed yields of progenies from High × High crosses were significantly superior to those from High × Low crosses. Apparently, the best seed yielding progeny plants came most frequently from outcrosses between two high seed yielding parents.

INTRODUCTION

Most researches in red clover have been directed toward improving its value as a forage crop. It is not surprising, therefore, that fertility problems have been neglected and that information on these problems is only fragmentary.

No published data were found dealing with the genetics of male sterility in red clover. In alfalfa, Childers (3) found that complete male sterility is caused by a duplicate factor mechanism and that the inheritance of partial male sterility can be attributed to three genes which are acting in a cumulative way to give a partially lethal effect. Julen (7), being unable to account for pollen sterility in alfalfa on the basis of meiotic irregularities, suggested that lethal genes must also occur and exert a depressing effect on pollen fertility. The presence of self-incompatibility complexes (S alleles) is a handicap in such genetic studies on red clover but Williams (13) overcame this to some extent by using sibcrosses to obtain the F_2. The same method was used by Starling *et al.* (9) to obtain F_2 populations.

The seed yielding ability in red clover is determined by the average values of seed weight, number of seeds per floret, number of florets per head, number of heads per plant and number of plants per unit area, all

[1] Contribution from the Departments of Genetics and Agronomy at Macdonald College, McGill University, based on a thesis submitted by B. Povilaitis to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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of which are affected to varying degrees by environment. Varieties owe their seed yielding abilities to different values for and combinations of these factors. According to Rudorf (8) the basic factors for seed production in red clover are the number of heads per plant and the number of florets per head. In order to estimate seed setting in red clover strains, Wilsie and Gilbert (14) counted the number of florets per head and the number of seeds per head and calculated the average percentage of florets setting seed.

Seed production is generally considered to be a genetically conditioned quantitative character. Jones (6) concluded that high seed production is not only genetically determined in alfalfa but also that the pattern of inheritance is in some respects much like that of resistance to some diseases.

Williams (13) found that fertility of cross-compatible plants of red clover does not appear to be depressed by inbreeding, but that during the early stages of growth the outbred seedlings were much more vigorous than the inbred ones. After about 8 weeks of growth the crossbreds were 18.6 per cent taller than F_2 seedlings, and 33.4 per cent taller than F_3 seedlings. Wexelsen (11) showed that outcrossing gives slightly more seed than inbreeding on the same plant. Torrie *et al.* (10) concluded that the forage yield of a synthetic variety of red clover is superior to that of the sibbed lines.

The present study was undertaken as a contribution toward a better understanding of fertility in diploid red clover.

MATERIALS AND METHODS

Two plants were selected for a study from the 1950 breeding nursery of Dollard red clover at Macdonald College. Since the object was to make crosses within and between their progenies, an effort was made to avoid complications with the S alleles by selecting each one from an apparently distinct line and by obtaining their progenies through open pollination. Also, one of the two plants (G) was selected to represent high seed yielding ability and the other (P) low. This rating as high and low was based on the following seed yields in grams per plant for 1950 and 1951: plant G, 12.1 and 8.2, respectively; plant P, 1.4 and 3.1, respectively. Yield data obtained since then, on clones of these two plants, in general confirm and support the above rating. Since, in addition, the 42 progeny (designated G1 to G42) from open pollination of plant G yielded an average of 90.7 seeds per head in 1952 as compared to 65.8 for 36 progeny (designated P1 to P36) from open pollination of plant P, the superiority of plant G seems well established.

The two sets of progenies from open pollination of the two selected plants, were grown for crossing and sibbing in 1952. In the summer of 1952 the crossing work was done in the laboratory following Battle's (1) method. In the winter of 1952-53 crosses were made on the same plants grown in pots in the greenhouse and the seeds were allowed to ripen on the plants. Progenies of a total of 18 crosses were then grown with clones of their 27 parental plants in the field in 1953. The seed yielding ability of the parental plants in 1952 and 1953, and of their progenies grown in 1953, was evaluated by counting the number of seeds produced per head.

It was decided that 20 heads per plant would constitute a representative sample but in some instances less than 20 mature heads per plant were available at the time of harvest.

The florets used for crosses were not emasculated. For pollination sharp pieces of cardboard were used, as described by Williams (12), as well as small pieces of very fine sandpaper glued on to toothpicks.

Lactophenol was found to be the best medium for mounting the pollen grains because it did not cause swelling, shrinkage, or bursting of the pollen grains as most other mounting media do. A small amount of fast green dye dissolved in the lactophenol stained the contents of the pollen green so that completely aborted and apparently normal grains were distinguishable. Three to four florets were tripped just before anthesis, the pollen placed in a drop of lactophenol on a slide and the coverslip applied. A pollen sample of about 600 grains, which was considered to be sufficiently large to be representative, was regularly examined. The pollen grains were classified as "normal" or "aborted" and counted when they appeared in the field of the microscope as the slide was moved in straight lines at random places.

Heads for the examination of meiosis from 7 plants, including the 2 originally selected, were fixed in Carnoy's 3:1:1. The squash preparations were stained in iron-aceto-carmine, or in leuco-basic fuchsin (hydrolysis time, 10 minutes) and counter-stained with fast green. Ovaries for embryo-sac studies were fixed in Craf fluid, sectioned at 10 μ and stained with iron haematoxylin.

RESULTS

Pollen Abortion

Parent plants used in all crosses were tested for pollen abortion several times in 1952 and again in 1953. There was little variation in the percentages of aborted pollen produced in the field at different times during the summer of 1952 by any one of the parental plants; e.g. 4.8, 3.4, 1.9, 3.5, 3.1 and 1.7 for plant G4 and 54.4, 51.0, 52.2, 53.7, 55.8 and 52.7 for plant P3. The means of aborted pollen of the parental plants for 1952 were very close to those obtained in 1953 and the correlation coefficient between the means for 1952 and 1953 was found to be positive, high in numerical value (0.966) and significant at the 0.001 point. These data suggest that each plant produces a typical proportion of aborted pollen. Mere comparison of the variation in proportion of aborted pollen within parental plants, with the variation between these plants, leads to the conclusion that the latter variation cannot be caused by environmental factors only or by errors of sampling. A fair assumption is that the differences are of genetic origin.

In 1953 the percentages of aborted pollen were determined for about 40 plants in each of 12 of the 18 progenies. Both parents of 4 of these progenies had a low percentage of aborted pollen; in 5 of the progenies one parent was low and the other high, and in the remaining 3 both parents had a high percentage of aborted pollen. A summary of results is given in Table 1.

TABLE 1.—MEAN PERCENTAGES OF ABORTED POLLEN IN THE PARENTS AND PROGENIES OF 12 CROSSES OF DIPLOID RED CLOVER

Cross No.	Parents				Progenies		
	Plants crossed		Mean per cent of aborted pollen in parents		Number of plants	Per cent aborted pollen	
	A	B	A	B		Mean	Range
112	G10	P15	4.4	2.2	32	3.9	0.6 – 24.4
15	G5	P13	3.8	3.5	40	5.2	0.9 – 48.3
5	G8	G10	4.4	4.7	29	6.0	1.1 – 26.0
17	G7	P9	10.4	11.8	33	11.2	1.4 – 52.1
18	G11	P12	22.3	6.3	40	9.1	1.5 – 47.9
3	G4	G9	2.4	31.9	40	31.7	1.4 – 89.3
21	G1	P1	3.3	39.0	40	14.7	1.0 – 46.2
16	G6	P2	3.7	48.4	36	32.0	2.9 – 48.4
20	G10	P14	4.4	28.5	49	14.8	0.9 – 53.9
8	P5	P7	28.9	44.1	40	32.5	3.8 – 64.6
19	G2	P3	36.1	50.7	40	29.0	1.9 – 61.2
9	P6	P8	40.1	39.1	40	35.7	0.8 – 80.1

The correlation coefficient between parental mean values and progeny means ($r = 0.894$) was positive and significant at the 0.001 point. Progeny means of most of the crosses were very close to the parental mean value (Table 1—crosses 5, 8, 9, 15, 16, 17, 20, 112), though some crosses produced progenies with means clearly higher (No. 3) or lower (No. 18, 19, 21) than the parental mean. Where one or both parents showed a high proportion of aborted pollen, the range in all the progenies was from a very low percentage of aborted pollen to higher than the mean of the highest parent. In progenies of all 12 crosses the range of variation was greater than the difference between the means of the corresponding parents. A remarkably wide range was observed in the progeny of cross 3 which was obtained from the cross of two sibs (Table 1), and also the distribution of percentages in this progeny was clearly bimodal.

Considerations of the above observations and of frequency distributions (not shown in Table 1) have led to the following conclusions:

(1) Progenies from crosses with both parents in the low aborted pollen group consist almost entirely of plants that can be placed in the same group as their parents, except for a very small proportion of plants that fall into more extreme groups.

(2) No matter what types of parents were used for the crosses nor what the central tendency of the progeny was, a certain varying proportion of plants in the progeny approached almost complete absence of aborted pollen. No completely male-sterile plants were found although occasional individual anthers were shrunken and filled with aborted pollen which they were unable to shed.

(3) The data suggest that the partial male sterility due to pollen abortion in diploid red clover is transmitted from parents to the progeny by means of some, as yet unexplained, genetic mechanism.

Ovule Abortion

It was noticed that when plant P14 was used as a seed parent, the percentage of florets setting seed was low (2.1 - 25.0) regardless of

TABLE 2.—PERCENTAGES OF SEED SET IN RECIPROCAL CROSSES BETWEEN PLANT P14 AND FIVE OTHER PLANTS

Female plant No.	Male plant No.	Number of florets pollinated	Percentage of florets setting seed
P14	P3	48	2.1
P14	G3	44	9.1
P14	G8	50	14.0
P14	G10	65	20.0
P14	P15	40	25.0
P3	P14	43	74.4
G3	P14	50	82.0
G8	P14	42	100.0
G10	P14	135	83.0
P15	P14	36	91.7

TABLE 3.—EMBRYO-SAC FAILURES IN THREE DIPLOID RED CLOVER PLANTS

Plant	Number of ovules sectioned	Per cent of ovules with no embryo sac
P14	60	51.7
G8	44	9.1
P3	48	4.2

pollen source whereas the pollen from this plant was highly successful in the reciprocal crosses, as shown in Table 2.

It was suspected that there might be something wrong with ovule development. To check this possibility ovaries of the plants G8, P3 and P14 were sectioned, stained and examined. The results can be seen in Table 3.

In about one-half of the ovules of plant P14 no embryo sacs were found, whereas the proportion was much smaller in the other two plants. This suggests that failure of embryo-sac development in certain plants is one cause of low seed set. This problem has been studied more extensively in 1954-55 and the results will be published at a later date.

Meiosis and Pollen Abortion

Meiosis was studied in the microsporocytes of the 7 plants previously noted. Only 2 metaphase I plates, out of a total of 190 examined, were aberrant: one contained one univalent, five bivalents and one trivalent; the other, two univalents and six bivalents. All of the remaining 188 plates had seven bivalents only. The first and the second divisions had very few irregularities of other kinds. In anaphase I lagging chromosomes, and in one plant 3 cells with bridges, were observed, but the total was only 1.75 per cent abnormal in 457 cells. A small proportion of abnormal cells in division II (1.99 per cent of 201 cells) and of quartets with supernumerary cells (2.39 per cent. of 1756) was also found. Thus, on the whole, the meiotic divisions were very regular in the 7 plants examined.

FIGURE 1. Frequency distributions of the number of seeds per head in all plants from 18 progenies of diploid red clover.

The percentages of aborted pollen in the 7 plants in which meiosis was studied were 1.8, 2.5, 3.3, 5.8, 28.5, 39.0, 50.7. The few abnormalities in meiosis might account for the low percentages of the first 4 plants but they cannot possibly account for all the aborted pollen in the last 3 plants listed. No previous data are available on this problem in red clover. In alfalfa Grun (4) and Julen (7) found no relationship between pollen abortion and meiotic irregulatities.

Seed Yield in the Progenies of Crosses

The progenies of the 18 crosses were grouped according to (1) the relationship of parents (outcrosses or sibcrosses), and (2) the seed yielding ability of the parents (progenies from crosses between high seed yielders and from crosses between high and low seed yielders). As a dividing-line for the grouping of the parent plants into High (H) and Low (L) seed yielding classes, an average of 73 seeds per head was taken, since this was the mean for the 27 parents in 1952. Plants yielding more than 73 seeds per head in either 1952 or 1953 were classed as "High," others as "Low". The groups of crosses, together with data on seed yield of both the parents and progenies, are shown in Table 4.

The correlation coefficient between parental mean values for seeds per head and the means of the progenies of all 18 crosses was positive and significant at the 0.001 point, r = 0.799 (D.F. - 16, r at 0.001 is 0.7084).

The general mean for the progenies of the sibbed group is 60.1 seeds per head and for the outcrossed group 81.4, and the difference is highly significant. As is shown in Figure 1, where the frequencies are presented in percentages within each group in order to obtain comparable curves, the ranges of all progeny plants in the sibbed and the outcrossed groups are almost the same, but the frequency distributions are quite different. Distribution of the outcrossed group is apparently normal, the only important departure being the small peak at the midpoint of 17.8 which was caused mainly by the progeny of one cross. The distribution of the sibbed group is far from normal, showing two major peaks, one at 10.7 and the other at 81.7.

In the outcrossed group the progeny means of 9 out of 10 crosses exceeded the parental mean value in number of seeds per head (Table 4 and Figure 2). Also, in the outcrossed group the mean yield of the progenies was significantly higher than the parental mean values in both High × High and High × Low categories. In the sibbed group the progeny means of 4 of the 8 crosses exceeded the parental mean value (Figure 2). The weighted mean yield of progenies of the whole sibbed group, and of the High × High and High × Low categories within this group, were each about 10 per cent lower than the corresponding parental mean value, and none of these differences was statistically significant.

In the High × High crosses, 6 out of 9 progenies exceeded the parental mean value in the number of seeds per head. In these crosses the parental mean values for the 4 sibbed progenies averaged nearly the same as for the 5 outcrossed ones, but, as mentioned above, the progenies from sibbed parents averaged lower in yield than either the parental mean values or the progeny means of the outcrossed parents. A similar situation existed where one parent was High and the other Low in seeds per head. In

TABLE 4.—MEAN NUMBER OF SEEDS PER HEAD AND PERTINENT STATISTICS FOR THE PROGENIES OF 18 CROSSES OF DIPLOID RED CLOVER

Cross No.	Parents: Plants crossed A	Parents: Plants crossed B	Type of cross	Seeds per head: A 1952	Seeds per head: A 1953	Seeds per head: B 1952	Seeds per head: B 1953	Mean	Progenies: Number of plants	Progenies: Mean	Progenies: C.V.[1]
Group from sibbed parents:											
7	P5	P15	H × H	96.0	70.1	104.5	91.6	90.5	15	99.7	13.91
5	G8	G10	H × H	83.1	41.1	31.5	81.3	59.2	24	84.9	19.43
6	P15	P10	H × H	104.5	91.6	99.0	113.7[2]	102.2	11	84.8	18.18
3	G4	G9	H × H	87.4	52.6	95.3	93.8[2]	82.3	39	57.7	65.47
			H × H					83.5	89	75.4[3]	
115	P15	P14	H × L	104.5	91.6	21.7	17.7	58.9	9	70.7	27.28
8	P5	P7	H × L	96.0	70.1	31.3	23.9[2]	55.3	38	58.8	57.24
10	P11	P4	H × L	73.6	71.8	29.7	32.6	51.9	30	42.0	68.07
9	P6	P8	H × L	91.7	58.8	35.1	16.7	50.6	40	38.6	80.12
			H × L					54.2	117	48.5[3]	
All sibcrosses								68.8	206	60.1[3]	
Group from outcrossed parents:											
112	G10	P15	H × H	31.5	81.3	104.5	91.6	77.2	30	100.3	16.49
15	G5	P13	H × H	91.6	75.1	79.4	89.5	83.9	40	96.8	20.71
109	G8	P15	H × H	83.1	41.1	104.5	91.6	80.1	28	93.0	20.48
14	G3	P15	H × H	118.5	78.8	104.5	91.6	98.3	15	90.7	14.00
16	G6	P2	H × H	81.2	66.6	63.0	74.5	71.3	35	86.3	16.45
			H × H					82.2	148	93.7[3]	
21	G1	P1	H × L	101.4	72.0	64.4	61.8	74.9	38	89.6	17.74
17	G7	P9	H × L	98.4	76.2	47.7	39.0	65.3	29	78.2	22.23
18	G11	P12	H × L	89.2	62.0	47.5	53.2	63.0	39	76.6	28.77
19	G2	P3	H × L	74.5	73.9[2]	60.7	36.2	60.9	40	62.4	21.08
20	G10	P14	H × L	31.5	81.3	21.7	17.7	38.0	47	58.7	51.97
			H × L					60.4	193	72.1[3]	
All outcrosses								71.3	341	81.4[3]	

[1] Coefficient of variation. [2] Missing values calculated on a least squares basis. [3] Weighted means.

general, the High × High crosses produced high yielding progenies more regularly when the parents were outcrossed than when they were sibbed.

The frequency distributions of plants with various mean numbers of seeds per head within each of the 18 progenies differed appreciably. Eight of the 9 progenies having apparently normal distributions came from outcrossing and only 1 from sibbing. Four of the 8 had both parents high seed yielding, and the other 4, one low and one high yielding parent. Two of the 3 progenies having apparently bimodal distributions came from sibbing. One of the two came from a High × High combination and the other from a High × Low one; the third came from a High × Low outcross. Two of the remaining 6 progenies had irregular distributions and the other 4 had too few plants to permit any statement about their frequency distributions. Coefficients of variation ranged from 13.91 to 80.12 for progenies from sibcrosses and from 14.00 to 51.97 for progenies from outcrosses. Progenies from sibcrosses were usually more variable in number of seeds per head than progenies obtained by outcrossing.

Pollen Abortion and Seed Set

The correlation coefficients between the number of seeds produced per head and the percentage of aborted pollen were calculated for 10 progenies as shown in Table 5. Only one of the coefficients was high and highly significant. This coefficient was obtained for the progeny of the sibcross (cross 3) which showed the most inbreeding depression in seed production (Figure 2). Frequency distributions for seed yield and for aborted pollen in this progeny are definitely bimodal. The high value of the correlation coefficient is caused in part by the abnormal distribution of this population. The latter indicates also that pollen abortion and seed set are genetically controlled in some way.

The correlation coefficient between the number of seeds per head and the per cent of aborted pollen in a pooled population of the above mentioned progenies for the total of 369 individuals was negative and significant at the 0.001 point ($r = -0.446$). No definite explanation can be advanced at present as to why the correlation coefficient between

TABLE 5.—CORRELATION COEFFICIENTS BETWEEN NUMBER OF SEEDS PER HEAD AND PERCENTAGE ABORTED POLLEN IN PROGENIES OF TEN CROSSES OF RED CLOVER

Cross No.	D.F.	r	C.D.[1]	r significant at level		
				.05	.01	.001
Group from sibbed parents:						
3	37	−0.881***	77.56			0.5073
9	38	−0.389*	15.14	0.3126	0.4032	
8	36	−0.144	2.07	0.3206		
5	22	0.122	1.48	0.4061		
Group from outcrossed parents:						
17	27	−0.423*	17.86	0.3683	0.4717	
18	37	−0.357*	12.74	0.3166	0.4082	
21	36	−0.345*	11.87	0.3206	0.4132	
16	33	−0.102	1.04	0.3347		
20	45	−0.086	0.47	0.2875		
19	38	0.083	0.69	0.3126		

[1] Coefficient of determination $-r^2 \times 100$.

percentage of aborted pollen and seed production per head is significant. These findings are compatible, however, with the idea that some of the genetic factors causing pollen abortion also cause ovule abortion to some extent.

Number of Florets per Head and Seed Set

Some data were obtained on the number of florets per head in the progenies of crosses 14, 20 and 21. The number of florets was counted on two heads per plant and the number of seeds produced in those heads under open pollination conditions was recorded. A complete summary of the data is given in Table 6.

TABLE 6.—RANGE AND MEAN NUMBER OF FLORETS AND OF OPEN POLLINATED SEEDS PER HEAD, AND THE CORRELATION COEFFICIENTS BETWEEN THE NUMBER OF FLORETS AND SEEDS PER HEAD IN THREE PROGENIES OF RED CLOVER

Cross No.	Number of Plants	Florets		Seed		r
		Mean	Range	Mean	Range	
14	15	123.5	84 – 196	90.7	58 – 138	0.898***
21	38	137.2	93 – 191	89.6	61 – 149	0.772***
20	47	120.5	76 – 180	58.7	5 – 120	0.395

The correlation coefficients between the number of florets and the number of seeds per head were significant at the 0.001 level for the two higher seed producing progenies and not significant for the low one. The present data show that the seed producing ability of a red clover plant, as measured by the number of seeds per head, cannot be accurately estimated by counting the number of florets per head when the plants involved are low yielding.

GENERAL DISCUSSION

The seed yields of plants of diploid red clover grown in 1953 varied greatly within progenies as well as between them. The range of variation of all plants in the 18 progenies was from 0.3 to 145.2 seeds per head*, that is from almost complete sterility to a very high seed set. The very low seed set in some plants cannot be attributed to self-incompatibility genes, for the plants were grown under conditions of open pollination. Also, such low yields are not the result of failures in pollination and fertilization caused by local variations in the environment since the seed sample at harvest time was quite large (seed from 20 heads, or, on the average, from 2000-3000 florets). Present evidence also eliminates abnormal chromosome behaviour in meiosis as the main and common cause of low seed set, providing that whenever meiosis is regular in pollen mother cells it is also regular in megaspore mother cells.

The results obtained in the present study show that one generation of inbreeding due to sibbing significantly reduces the number of seeds produced per head as compared with the outcrosses. Williams (13) found

* Povilaitis, B. Fertility in diploid and tetraploid red clover. Unpublished Ph.D. thesis, McGill University. 1954.

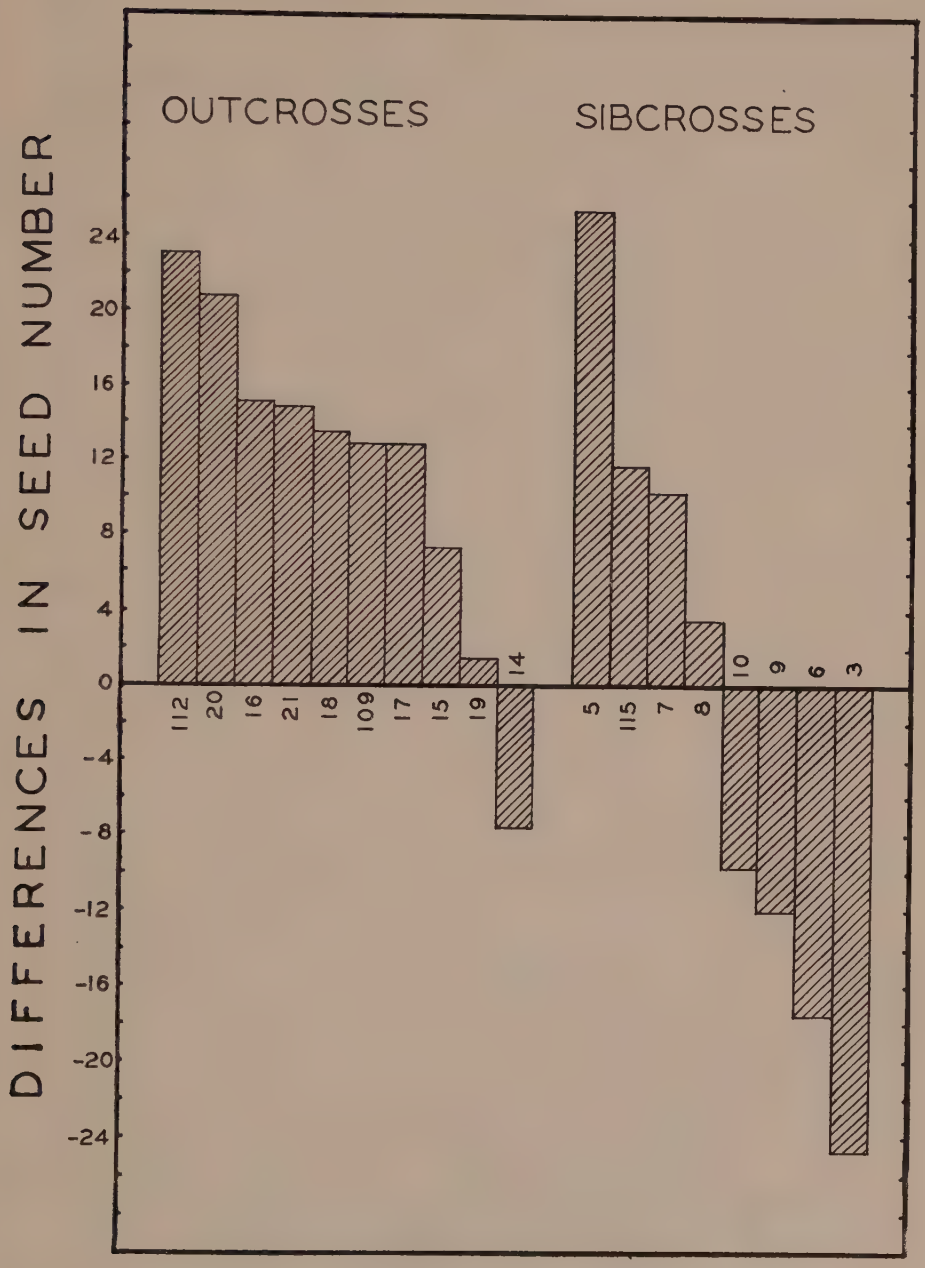

FIGURE 2. Differences in the number of seeds per head between the progeny means and the parental mean value of the 18 crosses of diploid red clover.

that fertility is in general not impaired by inbreeding red clover, but that vigour is; whereas, according to Wexelsen (11), it reduced the amount of seed per plant. Wexelsen (11) stated also that inbreeding has a drastic effect on plant weight at the flowering stage, and he found no line resistant to inbreeding depression. In our present study half of the progenies from sibcrosses showed inbreeding depression. Consideration of these points has led us to conclude that sibbing, even for only one generation, results in a depression of the seed yielding ability in some progenies of diploid red clover.

The means of the numbers of seeds per head for the outcrosses followed in a general way the mean parental value; the higher the mean parental value the higher the progeny mean. The progeny means of sibcrosses, both parents of which were good seed producers, were not always high. This is in agreement with Jones' (6) observation that high pod-producing populations of alfalfa were always the product of a cross between plants of recorded high seed production. The reverse was not always true since not all of his high seed producing plants gave uniformly high producing progeny when crossed. He also observed that the progenies of crosses between plants with high and low seed producing ability produced few or no high producing plants. This last observation of Jones is not in complete agreement with present findings in red clover, since at least some high seed yielding plants were present in the progenies of all High $\times$ Low crosses, whether sibcrossed or outcrossed.

Not much attention has been given by other investigators to the correlation between pollen abortion and seed production. Wexelsen (11) is of the opinion that poor pollen is evidently not of importance in the practical production of seed, but he did not correlate pollen abortion in the seed plant with its seed yielding ability. Hagberg (5) in *Galeopsis* and Bernström (2) in *Lamium* found a good correlation between "pollen fertility" and seed set, so that they felt justified in estimating the seed set on the basis of "pollen fertility". Nevertheless, Bernström casts doubt on the advisability of estimating seed set on the basis of "pollen fertility" alone, even in his material. Present results indicate that the proportion of pollen abortion is an important index of seed yield in progenies of certain crosses and that there is a limited general connection between seed yield and pollen abortion. It is probable that genetic factors control pollen abortion and possible that at least some of these genetic factors act to produce ovule abortion (see Table 2, plant P14) and in so doing reduce the amount of seed set.

The correlation coefficient between the means of number of seeds per head in the progenies of all 18 crosses and their corresponding parental mean values was positive and significant at the 0.001 level ($r = 0.799$). This indicates that the progeny means follow the parental mean values. By selecting high seed yielding parents and crossing them one increases the probability of obtaining a progeny of high mean seed yield.

It is certain that many different kinds of factors affect the amount of seed produced by diploid red clover plants. Environmental factors are known which operate before and after fertilization; the role of self-incompatibility genes is well known through the work of Williams and

others. The present study indicates that abnormal chromosome behaviour is of little importance in this connection, that important genetic factors exist which cause pollen abortion and perhaps also ovule abortion and that the amount of seed produced is affected through the action of these genes which can operate most effectively on the female side of reproduction. There may well be other genes which act at various stages of seed growth. Apparently these various genes have a cumulative effect, which amounts to a multiple gene action, operating in conjunction with the environmental factors, on the complex quantitative character which we call seed yield.

This study on diploid red clover has raised many questions but has left us with hope that the seed yielding ability of red clover varieties can be improved. It seems clear that by selecting plants which are high seed yielders under open pollination, and outcrossing them to other high yielding plants we can obtain some progenies which *on the average* yield as well as their parents and a few progenies that are even superior. By concentrating on removing the genetic weaknesses in those progenies and avoiding inbreeding, which depresses yielding ability, we should be able to produce better yielding varieties with all the genetic variability which seems necessary to maintain vigorous populations of diploid red clover.

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ENVIRONMENT AND POULTRY BREEDING PROBLEMS

III. THE PERFORMANCE OF 8 CROSSBRED AND 2 PUREBRED BROILER STRAINS AT THREE LOCATIONS

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ABSTRACT

Eight crossbred and 2 purebred "strains" of broilers, representing 8 commercial breeders and 2 experimental crosses, were raised at three farms, under a uniform diet and management routine.

Body measurements taken included 6-week body weight (at two locations) and body weight, breast angle, keel length and shank length at 10 weeks of age at all three locations. A sample of 60 birds of each "strain", with equal numbers of each sex, at each location was taken for securing body measurements, comprising a total of 1,800 birds.

The analysis of variance of the data, within sexes, showed a "strain" × location interaction for 6-week body weight in males and for breast angle for females at the P 0.05 level of significance. There was no other evidence of any important interactions between "strain" and location for the traits studied. Location effects were found to be statistically significant at the P 0.01 level of significance for all traits studied with the exception of 6-week body weights.

Strain effects for all traits measured at 10 weeks accounted for approximately 14 per cent and location effects for 10 per cent of the variance, despite the fact the rations and management procedures were standardized at all locations. Under the conditions of this experiment, the conclusion was reached that interactions between heredity and environment for the traits studied were of a low magnitude and thus relatively unimportant.

INTRODUCTION

The performance of birds of similar genotypes under different environments has important implications in poultry breeding and testing work. Although the importance of interactions between heredity and environment is widely appreciated in plant breeding, this is not the case in poultry breeding nor in animal breeding generally. Very little experimental work has been carried out on this problem of testing similar "strains" of birds representing different genotypes at different locations under what could be described as normal commercial broiler growing conditions, and thereby testing for the presence of genotype-environment interactions.

Tests of this nature are important also from the point of view of estimating the magnitude of location effects when standardized management practices and rations are used. These location effects as shown by Gowe and Wakely (5) for egg production at least, are quite large and they are generally not given the attention they deserve in evaluating performance, and in focusing attention on management and other factors which may be responsible for these location effects.

The Experimental Farms Service, with its system of branch farms, provided an opportunity to conduct an experiment to compare the performance of the same broiler strains at different locations and to test for the presence of interactions between heredity and environment.

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LITERATURE REVIEW

The general problem and the literature on the subject of interaction between heredity and environment in poultry has recently been reviewed by Gowe and Wakely (5). In general, there is a good deal of supporting evidence for interaction between heredity and environment for specific factors, such as nutritional deficiencies, Hutt (10), Howes and Hutt (8), Young (15); for resistance to diseases and parasites, Scholes and Hutt (14), Ackert *et al.* (1), Lerner *et al.* (12), Edgar *et al.* (2), Rosenberg *et al.* (13); and for heat resistance, Hutt (9), Fox (3).

Gutteridge and O'Neil (6), reported on the performance of three strains of Barred Plymouth Rocks raised at three locations. These workers found no evidence of an interaction between strain and location for the different traits studied but concluded that during the early period of growth the location effect was of greater magnitude than the strain effect. In their study no attempt was made to control the diet or management practices in a uniform manner over the three locations.

Gowe and Wakely (5) in their study of 2 pedigreed and 2 flock-mated strains of White Leghorns tested at six locations found no significant interaction between heredity and environment for mortality, and survivor and hen-housed egg production. In their study the diet, and all management practices, such as lights, floor space, and feeder space, were standardized over all locations. In spite of this control, the location effect was found to be very large and highly significant. However, more recently, Gowe (4) reported a strain environment interaction with Leghorn hens housed in individual laying cages and in floor pens.

MATERIALS AND METHODS

The breeds and crosses used in this study are shown in Table 1. These groups are referred to as "strains" in the rest of this report; and in the tables of the results code numbers are used to designate the strains.

Table 1.—Breeds and crosses used in test

Dominant Broad Breasted White No. 1 × New Hampshire
New Hampshire × Dominant White Wyandottes
(Red Cornish × New Hampshire) × Dominant White Peachblows
Dominant White meat strain × New Hampshire
Dominant Broad Breasted White No. 2 × New Hampshire
Columbian meat strain × (Rhode Island Red × New Hampshire)
Barred Plymouth Rock
(Dominant White Cornish × White Plymouth Rock) × Dominant White Plymouth Rock
Dominant White Plymouth Rock × Barred Plymouth Rock
White Plymouth Rock

The Broad Breasted White No. 1 and No. 2 are two strains of a breed which is being developed at Ottawa for meat production. Both of these strains were crossed with the same broiler strain of New Hampshires. The other 8 strains used in this test were obtained from commercial breeders in Canada and the United States, and were a sample of the "strains" being used in commercial broiler production at that time. The White Plymouth Rocks and Barred Plymouth Rocks were the only pure breeds represented, while all the other "strains" were either two-way or three-way crossbreds.

TABLE 2.—MEANS OF BODY MEASUREMENTS OF MALES AND FEMALES* OF 10 "STRAINS" AT 3 LOCATIONS

Sex	Body measurement	Location	Strain 1	2	3	4	5	6	7	8	9	10	Station mean
Males	6 wk. body weight (grams)	Ottawa											
		Lethbridge	797	781	811	820	758	769	779	705	738	733	769
		Charlottetown	807	770[1]	840	802	749	765	807	669	793	735	774
		Combined stations	802	775	825	811	753	767	793	687	766	734	771
	10 wk. body weight (grams)	Ottawa	1595	1520	1586	1535	1448	1511	1531	1309	1456	1442	1493
		Lethbridge	1650	1628	1645	1677	1561	1560	1603	1480	1531	1496	1583
		Charlottetown	1588	1504[1]	1606	1542	1479	1507	1572	1319	1530	1443	1509
		Combined stations	1611	1551	1612	1585	1496	1526	1569	1370	1506	1461	1528
	Breast angle (degrees)	Ottawa	80.3	80.5	76.3	79.2	78.2	79.2	77.3	78.0	81.7	76.5	78.7
		Lethbridge	83.2	84.5	79.0	82.7	80.8	81.5	80.2	81.8	82.7	78.2	81.5
		Charlottetown	82.7	83.3[1]	78.3	79.0	78.7	79.0	77.3	79.5	81.2	76.2	79.5
		Combined stations	82.1	82.8	77.9	80.3	79.2	79.9	78.3	79.8	81.8	76.9	79.9
	Keel length (cm.)	Ottawa	9.88	9.91	10.19	10.03	9.79	9.83	9.81	9.60	9.64	9.85	9.85
		Lethbridge	10.14	10.10	10.41	10.42	10.10	9.99	10.18	9.95	9.88	10.13	10.13
		Charlottetown	10.07	9.95[1]	10.48	10.33	10.16	10.10	10.30	9.77	10.15	10.16	10.15
		Combined stations	10.03	9.99	10.36	10.26	10.02	9.97	10.10	9.77	9.89	10.05	10.04
	Shank length (cm.)	Ottawa	11.21	11.13	11.40	11.34	11.05	11.12	11.34	10.75	10.95	11.02	11.13
		Lethbridge	11.10	11.17	11.42	11.38	11.23	11.16	11.31	10.86	10.99	11.03	11.17
		Charlottetown	10.77	10.69[1]	11.14	10.92	10.86	10.92	11.18	10.46	10.95	10.71	10.86
		Combined stations	11.03	11.00	11.32	11.21	11.04	11.07	11.28	10.69	10.96	10.92	11.05
Females	6 wk. body weight (grams)	Ottawa											
		Lethbridge	697	674	722	701	648	632	667	597	641	625	660
		Charlottetown	714	645	710	678	648	652[2]	673	583	619	635	656
		Combined stations	705	660	716	690	648	642	670	590	630	630	658
	10 wk. body weight (grams)	Ottawa	1265	1189	1247	1234	1196	1190[1]	1276	1069	1153	1159	1198
		Lethbridge	1352	1281	1348	1351	1270	1227	1296	1180	1292	1230	1283
		Charlottetown	1309	1186	1286	1222	1197	1193[2]	1245	1094	1139	1166	1204
		Combined stations	1309	1219	1294	1269	1221	1203	1272	1115	1195	1185	1228

TABLE 2.—MEANS OF BODY MEASUREMENTS OF MALES AND FEMALES* OF 10 "STRAINS" AT 3 LOCATIONS—*Concluded*

Sex	Body measurement	Location	Strain										Station mean
			1	2	3	4	5	6	7	8	9	10	
	Breast angle (degrees)	Ottawa	80.0	81.3	76.7	80.3	80.0	81.0[1]	78.2	77.3	80.2	76.8	79.2
		Lethbridge	84.8	84.5	80.7	81.8	82.3	81.7	78.8	80.0	82.0	79.2	81.6
		Charlottetown	81.7	82.8	80.0	80.2	79.8	81.0[2]	78.3	77.5	79.8	76.2	79.8
		Combined stations	82.2	82.9	79.1	80.8	80.7	81.2	78.4	78.3	80.7	77.4	80.2
	Keel length (cm.)	Ottawa	9.15	9.14	9.48	9.44	9.26	9.10[1]	9.21	8.89	8.81	9.14	9.16
		Lethbridge	9.46	9.36	9.72	9.72	9.34	9.32	9.34	9.23	9.16	9.34	9.40
		Charlottetown	9.47	9.29	9.74	9.46	9.42	9.29[2]	9.39	9.26	9.04	9.32	9.37
		Combined stations	9.36	9.26	9.65	9.54	9.34	9.24	9.31	9.13	9.00	9.27	9.31
	Shank length (cm.)	Ottawa	10.08	10.00	10.35	10.16	10.07	10.06[1]	10.36	9.82	9.97	10.00	10.09
		Lethbridge	10.01	10.00	10.32	10.26	9.96	10.02	10.20	9.85	10.16	9.96	10.07
		Charlottetown	9.94	9.73	10.06	9.85	9.67	9.75[2]	9.83	9.47	9.64	9.76	9.77
		Combined stations	10.01	9.91	10.24	10.09	9.90	9.94	10.13	9.71	9.92	9.91	9.98
Combined sexes	6 wk.	Combined stations	753	717	770	750	700	704	731	638	698	682	714
	10 wk.		1460	1385	1453	1427	1358	1364	1420	1242	1350	1323	1378
	Breast angle		82.1	82.8	78.5	80.5	79.9	80.5	78.3	79.0	81.2	77.1	80.0
	Keel		9.69	9.62	10.00	9.90	9.68	9.60	9.70	9.45	9.44	9.66	9.67
	Shank		10.52	10.45	10.78	10.65	10.47	10.50	10.70	10.20	10.44	10.41	10.51

*No. of birds 30 per strain per sex per station except in 3 cases noted.

[1]Mean based on 29 birds.

[2]Mean based on 26 birds.

One case of eggs was obtained from each of the commercial breeders represented. All eggs were incubated at Ottawa in a completely randomized manner in the incubators. The chicks were wing-banded and assigned at random within strain to the three locations: Ottawa, Ontario; Lethbridge, Alberta; and Charlottetown, P.E.I. The chicks were randomized in the shipping-boxes and shipped by air to Lethbridge and Charlottetown on the date of hatch, April 20, 1955. A total of 2,787 chicks were placed on test at the three locations.

The same feeding program was used at all three locations. A 22 per cent protein broiler mash was fed *ad libitum* to 8 weeks of age. This ration was mixed by a commercial feed company, to our formula, at an eastern and western mixing plant of the company. Ottawa and Charlottetown were supplied by the eastern mixing plant and Lethbridge by the western mixing plant. At 8 weeks, this ration was mixed, at each station, with ground wheat to give an 18 per cent protein ration.

All management practices were standardized, as far as possible, at the three locations. Floor space per bird was 1 square foot from date of hatch to 10 weeks. All birds were raised in complete confinement, and uniform standards were maintained for feeding and watering space. There was no separation of sexes throughout the test.

No vaccination for any disease was carried out at any of the locations. Sulphaquinoxaline was included in the ration at the preventive level (.0125 per cent) for the control of coccidiosis.

There was no culling for any reason throughout the test, except that crippled chicks were eliminated at hatching.

The following body measurements were taken: Body weight at 6 weeks (at two locations only); and body weight, breast angle, keel length and shank length at 10 weeks. Body weight and breast angle were determined on the live birds, keel length and shank length on the dressed carcasses. Body weights were taken to the nearest 10 grams. Breast angle was taken with a modified "West Virginia Breast Angle Meter" and measured to the nearest 5 degrees. Outside calipers were employed to measure keel and shank length and measurements were taken to the nearest millimetre. Other measurements and grades were also obtained on dressed birds but these are not included in this report.

Due to the limitation of the plant facilities at the stations represented, only 60 birds of each "strain", with equal numbers of each sex, were slaughtered at each station. This was done so that all birds could be slaughtered on the one day. The birds to be slaughtered were selected in a completely random manner without any attention whatsoever paid to the appearance of the bird or its previous live bird record.

Only the data obtained on the birds which were slaughtered have been used in the analyses and the tables of means.

RESULTS AND DISCUSSION

The mortality of all birds raised at the three locations was 2.8 per cent to 10 weeks of age, the percentages by station being: Ottawa, 2.2; Lethbridge, 3.3; Charlottetown, 2.9. There was very little difference in mortality between strains.

TABLE 3.—ANALYSES OF VARIANCE OF BODY MEASUREMENTS OF 10 "STRAINS" AT 3 LOCATIONS

Source	D.F.	Mean square 6 wk. wt.	D.F.	Mean square 10 wk. wt.	Mean square breast angle	Mean square keel length	Mean square shank length
Males							
Location	1	3,082	2	689,998**	593.20**	8.18**	8.38**
Strains	9	98,748**	9	504,906**	325.56**	2.54**	3.09**
Location × strains	9	10,780*	18	25,307	16.75	0.23	0.22
Remainder	579	5,544	869	21,512	12.23	0.23	0.24
Females							
Location	1	3,362	2	674,677**	473.27**	4.95**	9.67**
Strains	9	89,040**	9	310,472**	288.45**	3.07**	1.97**
Location × strains	9	4,591	18	16,702	21.62*	0.14	0.18
Remainder	576	4,286	865	13,305	11.32	0.21	0.19

*Significant at 5% level.
**Significant at 1% level.

Table 2 shows the means of the body measurements by sex, strain, and location and includes an over-all summary showing combined sexes and locations. There were three cases in which the sample size did not reach 30 birds and these are indicated in the table.

The analyses of variance for males and females separately for the different body measurements are presented in Table 3. In those cases in which the sample size did not reach 30 birds, the analyses were completed on the basis of 30 by the substitution of mean values. Allowance is made for this substitution in the degrees of freedom associated with the "remainder" source of variation. Six mean substitutions were made in all.

In the 6-week weights, where there were only two locations involved, the differences between the means for stations for males and females were 5 grams and 4 grams respectively. The body weight range for the 10 strains was 138 grams for the males and 126 grams for the females. In the analyses of variance, for both males and females, there was no evidence of a location effect, although the strain × location interaction was significant at P 0.05 level for the males but not for the females. The strain effect was significant at P 0.01 level for both males and females.

The range in mean body weight at 10 weeks between stations was 90 grams for males and 85 grams for females, while the range of strain means was 242 grams for males and 194 grams for females. The location and strain effects were significant at the P 0.01 level for males and females, but the interaction was not significant. Location and strain effects were also significant at the P 0.01 level for both males and females for the other body measurements, namely, breast angle, keel length and shank length, but the only significant interaction (P 0.05) was for breast angle in the females.

TABLE 4.—PERCENTAGE OF THE TOTAL VARIANCE ASSOCIATED WITH LOCATIONS, STRAINS AND INTERACTION OF LOCATION AND STRAIN*

Source	6 wk. wt.	10 wk. wt.	Breast angle	Keel length	Shank length
Males					
Location	0	8	11	10	10
Strains	21	18	19	10	10
L & S	2	<1	<1	0	0
Remainder	77	74	69	80	80
Females					
Location	0	12	9	8	13
Strains	25	17	18	11	8
L & S	<1	<1	2	0	0
Remainder	75	70	71	81	79

*Negative components of variance were treated as zero.

The percentages of the total variance associated with locations, strains, the interaction of strains and locations and for individuals are presented in Table 4. In order to estimate the variances associated with the main effects and interactions, both locations and strains were considered to be random variables.

With the sample of locations and strains represented in this study, the variance associated with locations was less than that associated with strains for all the traits studied. The variance associated with the interaction of genotypes and environments was 2 per cent of the total in two cases while it was less than 1 per cent or zero in all other cases.

Of interest in this study are the two interactions (6-week body weights for males and breast angle for females), which were found to be of statistical significance. For the 6-week weights only two locations were represented and the over-all means of these two locations were very close showing a difference of only 5 grams. This is rather close agreement, and this is reflected in the mean square for locations being less than that associated with the remainder mean square. For most breast angle measurements, the operator effect is rather large, and even though the repeatability of measurements by a single operator may be quite good, the possibility exists that this interaction may represent, in part, an operator and strain interaction.

As pointed out by Haldane (7), a number of different types of interaction between heredity and environment may exist. However, as indicated by Lerner (11), interactions of a non-linear type are of chief importance from the standpoint of applied breeding and testing plans. Although it is possible to demonstrate interactions between heredity and environment when environmental conditions are rather extreme (*literature cited*), the problem as pointed out by Gowe and Wakely (5) from a practical breeder's standpoint is whether important interactions exist under commercial conditions where diets are considered adequate and good commercial management and disease prevention measures are followed. There is some evidence that under certain commercial practices, such as floor pens vs. laying batteries, interactions may exist and be of some importance (Gowe, 4). However, there is no evidence under the conditions of this experiment that interactions between "strains" and locations are important. Hence there would seem to be no need to specify specific strains for specific locations, although it might be desirable to test this over a greater range of environments, more widely representative of commercial conditions. However, it would appear logical to assume that, if interactions were found to be of some importance under the conditions of this experiment, they would be of even greater importance under more widely varying environments.

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